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ROZPRAWA DOKTORSKA

w formie spójnego tematycznie cyklu artykułów opublikowanych w czasopismach naukowych

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Mikroekstrakcja w zatężaniu i oznaczaniu śladowych ilości pierwiastków technikami rentgenowskiej spektrometrii fluorescencyjnej

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SPIS TREŚCI

1. SPIS PUBLIKACJI BĘDĄCYCH PODSTAWĄ ROZPRAWY DOKTORSKIEJ

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2. STRESZCZENIE

Współcześnie stosowane techniki analityczne pozwalają na wykrywanie i oznaczanie analitów na poziomie śladowym bądź ultraśladowym. Często jednak bezpośrednia analiza jest utrudniona bądź wręcz niemożliwa ze względu na zbyt niskie stężenie analitu bądź matrycę próbki charakteryzującą się złożonym składem. W takich przypadkach konieczna jest wstępna izolacja lub/i wzbogacanie analitów. Wśród najczęściej stosowanych technik pozwalających przygotować próbki do pomiaru szczególne miejsce zajmują techniki ekstrakcyjne, w tym ekstrakcja typu ciecz-ciecz (LLE) oraz ekstrakcja do fazy stałej (SPE). Wady klasycznych technik ekstrakcyjnych związane są głównie ze stosowaniem w nich dużych ilości szkodliwych dla zdrowia i środowiska przyrodniczego rozpuszczalników organicznych. Alternatywą jest stosowanie technik mikroekstrakcyjnych, w których analizuje się próbki o niewielkich objętościach, zużywając przy tym niewielkie ilości rozpuszczalników organicznych i generując małe ilości odpadów. W ramach niniejszej rozprawy doktorskiej opracowano procedury analityczne umożliwiające wzbogacanie i oznaczanie szeregu pierwiastków śladowych z zastosowaniem rentgenowskiej spektrometrii fluorescencyjnej (XRF). Zaproponowano procedury analityczne oparte na:

- **DLLME/EDXRF** - dyspersyjnej mikroekstrakcji typu ciecz-ciecz oraz rentgenowskiej spektrometrii fluorescencyjnej z dyspersją energii do jednoczesnego oznaczania jonów Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Ga(III), Se(IV) oraz Pb(II) z zastosowaniem pirolidynoditiokarbaminian amonu (APDC) oraz dietyloditiokarbaminianu sodu (Na-DDTC) jako odczynników chelatujących,
- **DMSPE/TXRF** - dyspersyjnej ekstrakcji do mikro-fazy stałej oraz rentgenowskiej spektrometrii fluorescencyjnej z całkowitym odbiciem promieniowania do oznaczania jonów Cd(II) oraz Pb(II) z zastosowaniem wielościennych nanorurek węglowych (MWCNT) jako adsorbentu,
- **DMSPE/EDXRF** - dyspersyjnej ekstrakcji do mikro-fazy stałej oraz rentgenowskiej spektrometrii fluorescencyjnej z dyspersją energii do jednoczesnego oznaczania jonów Co(II), Cu(II), Ni(II) i Pb(II), a także analizy specjacyjnej Se, po zatężaniu na powierzchni grafenu.

W celu uzyskania najkorzystniejszego stosunku sygnału do tła dla każdej z zaproponowanych procedur opracowano sposób nanoszenia próbki otrzymanej po procesie zatężania na odpowiednie podłoże. Dla uzyskania najlepszego odzysku oznaczanych pierwiastków sprawdzono wpływ takich parametrów jak: pH analizowanego roztworu, ilość odczynnika chelatującego (APDC lub Na-DDTC), objętość analizowanego roztworu, objętość ekstrahentu/adsorbentu oraz czas prowadzenia ekstrakcji/adsorpcji. Zaproponowane procedury analityczne pozwoliły na uzyskanie bardzo niskich granic wykrywalności: (i) DLLME/EDXRF - od 1,6 do 4,1 ng mL^{-1} dla próbki o objętości 5 mL, (ii) DMSPE/TXRF - od 1,0 do 2,1 ng mL^{-1} (20 mL próbki), (iii) DMSPE/EDXRF - od 0,23 do 1,1 ng mL^{-1} (50 mL próbki) oraz 0,032 ng mL^{-1} w przypadku analizy specjacyjnej Se (75 mL próbki). Uzyskanie tak niskich granic wykrywalności możliwe było dzięki wysokim wartościom współczynników wzbogacenia dla poszczególnych procedur: DLLME/EDXRF - 250, DMSPE/TXRF - 40 oraz DMSPE/EDXRF - od 418 do 2553.

Równoczesny pomiar zawartości pierwiastków wchodzących w skład próbki, niskie granice wykrywalności, krótki czas wykonania i niskie koszty analizy, czynią z zaproponowanych procedur obiecujące narzędzie umożliwiające oznaczanie zawartości wielu pierwiastków w jednej próbce. Zastosowanie technik ekstrakcyjnych pozwala ponad 1000-krotne obniżenie granic wykrywalności a tym samym na rozszerzenie zastosowania spektrometrii XRF o analizę próbek ciekłych.

2. SUMMARY

Nowadays, commonly applied analytical techniques allow determination of analytes on trace or ultratrace levels. However, the direct analysis of sample is not always possible due to low concentrations of analytes or too complicated matrix. Therefore, prior isolation and/or preconcentration step is necessary before the analysis. The one of the most popular sample pretreatment methodologies are extraction techniques such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE). In order to eliminate the disadvantages of the classical extraction techniques, associated mainly with use of large amounts of organic solvents, microextraction techniques based on miniaturization of classical extraction modes are introduced. In microextraction techniques small sample volumes are analysed, therefore the volume of organic solvent is reduced and, in consequence, the amount of generated organic waste decreases. The aim of the PhD thesis is development of the analytical procedures enabling preconcentration and determination of a number of trace elements using X-ray fluorescence (XRF) techniques. The proposed procedures combine:

- **DLLME/EDXRF** - dispersive liquid-liquid microextraction and energy-dispersive X-ray fluorescence spectrometry (EDXRF) for simultaneous determination of Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Ga(III), Se(IV) and Pb(II) ions with the use of ammonium pyrrolidinedithiocarbamate (APDC) and sodium diethyldithiocarbamate (Na-DDTC) as chelating agents and carbon tetrachloride as an extraction solvent,
- **DMSPE/TXRF** - dispersive micro-solid phase extraction and total-reflection X-ray fluorescence spectrometry for determination of Cd(II) and Pb(II) ions with multiwalled carbon nanotubes MWCNTs as an adsorbent,
- **DMSPE/EDXRF** - dispersive micro-solid phase extraction and energy-dispersive X-ray fluorescence spectrometry (EDXRF) for simultaneous determination of Co(II), Cu(II), Ni(II) and Pb(II) ions and speciation of Se, with graphene applied as an adsorbent.

In order to obtain the best signal to background ratio, different modes of sample deposition onto the sample carrier were evaluated. Moreover, various parameters affecting recovery of extracted elements such as the sample pH, the amount of chelating agent (APDC or Na-DDTC), sample volume, the volume of extraction solvent/adsorbent and extraction/adsorption time were investigated. The proposed procedures enable very low detection limits to be obtained: (i) DLLME/EDXRF - from 1.6 to 4.1 ng mL^{-1} for 5 mL samples, (ii) DMSPE/TXRF - from 1.0 to 2.1 ng mL^{-1} for Pb (20 mL samples), (iii) DMSPE/EDXRF - from 0.23 to 1.1 ng mL^{-1} (for 50 mL samples) and 0.032 ng mL^{-1} for speciation of Se (for 75 mL samples). It is noteworthy that receiving such low detection limits was possible to obtain due to high enrichment factor values: DLLME/EDXRF - 250, DMSPE/TXRF - 40 and DMSPE/EDXRF - 418 (Pb) - 2553 (Cu).

The proposed procedures are promising tools in multielemental analysis due to the possibility of simultaneous determination of enriched elements, low detection limits, simplicity, rapidity and low costs. Moreover, microextraction procedures extend the scope of XRF usage to liquid samples analysis and enable decreasing obtained detection limits over 1000 times.

3. Spis skrótów stosowanych w pracy

Skrót	Termin polskojęzyczny	Termin anglojęzyczny
APDC	Pirolidynoditiokarbaminian amonu	Ammonium pyrrolidinedithiocarbamate
ASV	Anodowa woltamperometria stripingowa	Anodic stripping voltammetry
CFME	Przepływowa mikroekstrakcja typu ciecz-ciecz	Continuous-flow microextraction
CIAME	Mikroekstrakcja za pomocą rozpuszczalnika poprzez ochłodzenie próbki	Cold-induced aggregation microextraction
CME	Mikroekstrakcja kapilarna	Capillary microextraction
DLLME	Dyspersyjna mikroekstrakcja typu ciecz-ciecz	Dispersive liquid-liquid microextraction
DMSPE	Dyspersyjna ekstrakcja do mikro-fazy stałej	Dispersive micro-solid phase extraction
EDXRF	Rentgenowska spektrometria fluorescencyjna z dyspersją energii	Energy-dispersive X-ray fluorescence spectrometry
EPA	Amerykańska Agencja Ochrony Środowiska	United States Environmental Protection Agency
ET-AAS	Atomowa spektrometria absorpcyjna z atomizacją elektrotermiczną	Electrothermal atomic absorption spectrometry
ETV-ICP-MS	Spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie z elektrotermicznym odparowaniem próbki	Electrothermal vaporization inductively coupled plasma mass spectrometry
F-AAS	Atomowa spektrometria absorpcyjna z atomizacją w płomieniu	Flame atomic absorption spectrometry
FCCCD	Centralny plan kompozycyjny z parametrem α=1	Face-cantered central composite design
GC-MS	Chromatografia gazowa ze spektrometrią mas	Gas chromatography-mass spectrometry
HF-LPME	Mikroekstrakcja poprzez membranę do fazy ciekłej	Hollow fiber-liquid phase microextraction
HF-SPME	Mikroekstrakcja poprzez membranę do fazy stałej	Hollow fiber-solid phase microextraction
HPLC	Wysokosprawna chromatografia cieczowa	High performance liquid chromatography
HR-ICP-MS	Wysokorozdzielcza spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie	High resolution inductively coupled plasma mass spectrometry
HS-SDME	Mikroekstrakcja do pojedynczej kropli fazy ciekłej z fazy nadpowierzchniowej	Headspace-single drop microextraction
HS-SPME	Mikroekstrakcja do fazy stacjonarnej z fazy nadpowierzchniowej	Headspace solid-phase micro extraction
ICP-MS	Spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie	Inductively coupled plasma mass spectrometry
ICP-OES	Atomowa spektrometria optyczna ze wzbudzeniem w plazmie sprzężonej indukcyjne	Inductively coupled plasma optical emission spectrometry
LA-ICP-MS	Spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie z ablacją laserową	Laser ablation inductively coupled plasma mass spectrometry
LIBS	Laserowo indukowana spektroskopia emisyjna	Laser induced breakdown spectroscopy
LLE	Ekstrakcja ciecz-ciecz	Liquid-liquid extraction
LLLME	Mikroekstrakcja do pojedynczej kropli w układzie "kropla do kropli"	Liquid-liquid-liquid microextraction
LOD	Granica wykrywalności	Limit of detection
LPME	Mikroekstrakcja do fazy ciekłej	Liquid phase microextraction
MSPME	Membranowa mikroekstrakcja do fazy stacjonarnej	Membrane solid-phase microextraction

MWCNT	Wielościenne nanorurki węglowe	Multi-wall carbon nanotubes
Na-DDTC	Dietyloditiokarbaminian sodu	Sodium diethyldithiocarbamate
SDME	Mikroekstrakcja do pojedynczej kropli ekstrahentu	Single drop microextraction
SFODME	Mikroekstrakcja do pływającej na powierzchni roztworu kropli rozpuszczalnika organicznego	Solidified floating organic drop microextraction
SPE	Ekstrakcja do fazy stałej	Solid phase extraction
SPME	Mikroekstrakcja do fazy stacjonarnej	Solid phase microextraction
TXRF	Rentgenowska spektrometria fluorescencyjna z całkowitym odbiciem promieniowania	Total-reflection X-ray fluorescence spectrometry
USAEME	Mikroekstrakcja poprzez emulgację wspomagana ultradźwiękami	Ultrasound-assisted emulsification-microextraction
WDXRF	Rentgenowska spektrometria fluorescencyjna z dyspersją długości fali	Wavelength dispersive X-ray fluorescence
XRF	Rentgenowska spektrometria fluorescencyjna	X-ray fluorescence

4. WSTĘP

Wprowadzenie nowych rozwiązań technicznych pozwoliło na udoskonalenie dotychczas stosowanej aparatury pomiarowej dzięki czemu możliwe jest oznaczanie analitów na poziomie śladowym bądź ultraśladowym. Bezpośrednia analiza próbek biologicznych lub środowiskowych nadal jednak nastręcza wielu trudności wynikających głównie ze zbyt niskiego poziomu stężeń analitów bądź skomplikowanej matrycy próbek. Z tego względu oznaczanie analitów w tego typu próbkach zwykle poprzedzone jest wstępną izolacją lub/i wzbogacaniem analitów. Spośród szerokiego wachlarza technik rozdziału opartych m.in. na strącaniu i współstrącaniu, odparowaniu i destylacji, ekstrakcji, elektroosadzaniu, sorpcji czy wymianie jonowej, w ostatnich latach największym zainteresowaniem cieszą się techniki ekstrakcyjne typu ciecz-ciecz (LLE) oraz ciecz-ciało stałe (SPE). Pomimo dużej popularności klasycznej techniki LLE, jej wieloetapowy charakter sprawia, że może być uznana za czasochłonną i skomplikowaną. SPE rozwiązuje wiele problemów występujących w LLE, takich jak: niecałkowite rozdzielanie faz, zużywanie dużych ilości rozpuszczalników organicznych czy trudność w automatyzacji. SPE jest jednak również procedurą wieloetapową i czasochłonną, ograniczoną w dużej mierze przez natężenie przepływu strumienia cieczy przez złoże. Ponadto żadna z klasycznych technik ekstrakcyjnych nie spełnia podstawowych zasad „zielonej chemii", której celem jest m.in.:

- znaczne ograniczenie zużycia szkodliwych rozpuszczalników organicznych,
- zmniejszenie emisji par, gazów, odpadów stałych i ścieków,
- ograniczenie lub wyeliminowanie toksycznych odczynników,
- wprowadzanie instrumentalnych metod analizy,
- zmniejszenie pracochłonności i energochłonności danego procesu [1,2].

W powyższy trend wpisuje się mikroekstrakcja typu ciecz-ciecz oraz mikroekstrakcja do fazy stałej. W technikach tych analizuje się próbki o niewielkich objętościach, zużywając przy tym nieznaczne ilości odczynników chemicznych i generując małe ilości odpadów. Podstawowe założenia procedur mikroekstrakcyjnych sprawiają, że można je rozpatrywać jako proste, szybkie, przyjazne dla środowiska naturalnego i stanowiące konkurencję dla klasycznych technik ekstrakcyjnych.

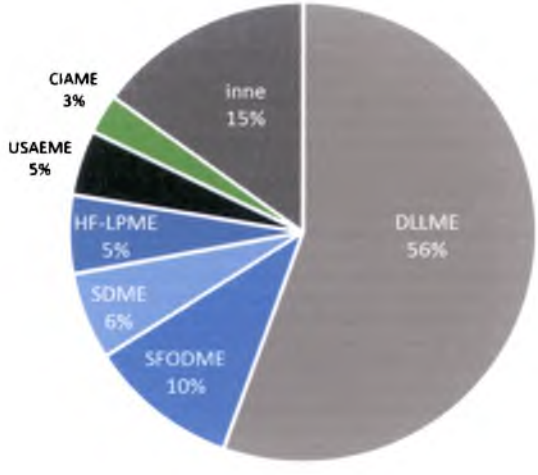
4.1. MIKROEKSTRAKCJA DO FAZY CIEKŁEJ

Mikroekstrakcja do fazy ciekłej (LPME) jest obecnie jedną z najintensywniej rozwijanych technik zatężenia i/lub izolacji analitów, a w literaturze można znaleźć wiele jej wariantów. W mikroekstrakcji do pojedynczej kropli (SDME) niewielka objętość odczynnika organicznego znajduje się na końcu kapilary [3] lub teflonowego włókna [4]. Ekstrahent może być bezpośrednio zanurzony w analizowanym roztworze (przepływowa mikroekstrakcja typu ciecz-ciecz, CFME) [5], zawieszony nad próbką (mikroekstrakcja do pojedynczej kropli fazy ciekłej z fazy nadpowierzchniowej, HS-SDME) [6-8] lub w postaci cienkiej warstwy pływać po powierzchni próbki (mikroekstrakcja do pojedynczej kropli w układzie "kropla do kropli", LLLME) [9]. Zastosowanie SDME pozwala na zmniejszenie objętości zużywanych odczynników do kilkunastu mikrolitrów. Mankamentem tej techniki jest brak stabilności mikro-kropli ekstrahenta. Problem ten rozwiązano m.in. przez zastosowanie włókien porowatych w tzw. mikroekstrakcji poprzez membranę do fazy ciekłej (HF-LPME). W metodzie tej ekstrahent znajduje się wewnątrz hydrofobowego, porowatego włókna, wykonanego najczęściej z polipropylenu. Dzięki działaniu sił kapilarnych ekstrahent wypełnia zarówno pory włókna jak i jego wnętrze. W zależności od właściwości rozpuszczalników wypełniających wnętrze włókna i jego porów wyróżnia się dwufazową [10] i trójfazową [11] wersję HF-LPME. Wprowadzenie hydrofobowych włókien umożliwiło zwiększenie szybkości mieszania próbki, co znacznie poprawiło kinetykę i wydajność ekstrakcji. Ponadto niewielkie rozmiary porów pozwalają na mikrofiltrację analizowanego roztworu uniemożliwiając przenikanie większych cząstek do wnętrza włókna. Dzięki temu możliwe jest analizowanie próbek o złożonych matrycach, w tym próbek biologicznych i środowiskowych. Kolejnym często stosowanym typem LPME jest mikroekstrakcja do pływającej na powierzchni roztworu kropli rozpuszczalnika organicznego (SFODME) [12]. W SFODME dla skrócenia czasu ekstrakcji badany roztwór jest mieszany mechanicznie lub przy użyciu ultradźwięków. Po przeprowadzeniu procesu zatężania próbkę umieszcza się w łaźni lodowej, gdzie po zestaleniu, wzbogacona faza organiczna może być łatwo usunięta z analizowanego roztworu i roztopiona przed pomiarem. Ekstrahent stosowany w SFODME powinien mieć temperaturę topnienia zbliżoną do temperatury pokojowej (10-30°C), gęstość mniejszą od wody i być w niej nierozpuszczalny.

Techniką mikroekstrakcyjną stosowaną zdecydowanie najczęściej w zatężaniu śladowych ilości jonów metali ciężkich jest dyspersyjna mikroekstrakcja w układzie ciecz-ciecz (DLLME). W technice tej do próbki energicznie wstrzykuje się mieszaninę rozpuszczalników: (i) ekstrakcyjnego (najczęściej halogenowany węglowodór, np. tetrachlorek węgla, chloroform, tetrachloroetylen, chlorobenzen) oraz (ii) dyspersyjnego (np. aceton, acetonitryl, etanol lub metanol). Sprzyja to formowaniu się mikro-kropel rozpuszczalnika ekstrahującego rozproszonych w całej objętości analizowanego roztworu, dzięki czemu powierzchnia kontaktu pomiędzy ekstrahentem a próbką wodną jest bardzo duża. W konsekwencji proces ekstrakcji jest praktycznie niezależny od czasu. Procedura DLLME składa się z dwóch etapów: (i) wstrzyknięcia mieszaniny rozpuszczalnika ekstrahującego i dyspersyjnego do fazy wodnej,

oraz (ii) odwirowania mętnego roztworu. Wydajność procesu ekstrakcji zależy przede wszystkim od rodzaju i objętości zastosowanego ekstrahenta oraz rozpuszczalnika dyspersyjnego. Ekstrahent w DLLME powinien mieć gęstość większą od gęstości wody, umożliwiać ekstrakcję analitów oraz pomiar wybraną techniką instrumentalną. Warto zaznaczyć, że wzrost objętości rozpuszczalnika ekstrahującego prowadzi do spadku wartości współczynnika wzbogacenia. Optymalna objętość odczynnika ekstrahującego powinna być jednak na tyle duża, by wystarczyła do przeprowadzenia pomiaru. Ilość zastosowanego odczynnika dyspersyjnego wpływa na stopień dyspersji ekstrahenta w analizowanym roztworze, a przez to bezpośrednio na wydajność procesu ekstrakcji. Procedura DLLME jest prosta w wykonaniu, szybka, tania, pozwala na uzyskanie wysokiego odzysku i wysokich współczynników wzbogacenia. Technika ta jest jednak trudna do zautomatyzowania i tylko niewielka liczba odczynników organicznych spełnia kryteria postawione ekstrahentom. Dla polepszenia efektywności DLLME wprowadzono m.in. dodatek środka powierzchniowo czynnego, zastosowano ultradźwięki, intensywne mieszanie próbki lub jej sekwencyjne dozowanie. Inny sposób modyfikacji tej techniki polega na wprowadzaniu cieczy jonowych, rozpuszczalników o gęstości mniejszej od gęstości wody lub koacerwatów i odwróconych miceli.

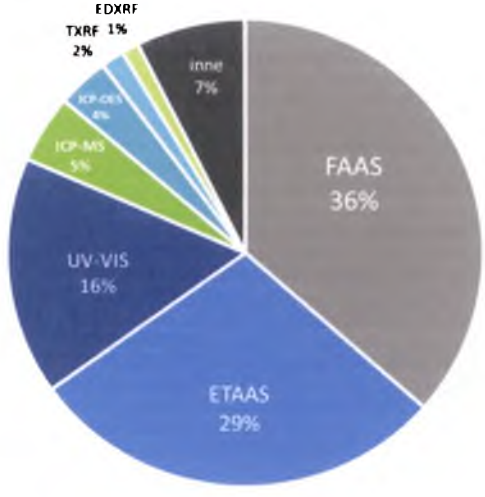
Początkowo techniki mikroekstrakcyjne były stosowane do oznaczania związków organicznych z zastosowaniem chromatografii gazowej ze spektrometrią mas (GC-MS) oraz wysokosprawnej chromatografii cieczowej (HPLC) [13,14]. W 2003 roku po raz pierwszy zastosowano LPME w połączeniu z absorpcyjną spektrometrią atomową z atomizacją elektrotermiczną (ET-AAS) do oznaczania jonów As(III) [15]. Od tego czasu liczba publikacji dedykowanych analizie nieorganicznej i specjacji metali stale rośnie. Najczęściej stosowane w analizie nieorganicznej rodzaje LPME przedstawiono na rys. 1.



Rys. 1. LPME w oznaczaniu śladowych ilości jonów metali ciężkich. Wyjaśnienia akronimów przedstawiono na stronach 7-8.

Techniki pomiarowe stosowane w oznaczaniu jonów metali zatężanych metodami DLLME. Wybór odpowiedniej metody analitycznej pozwala na uzyskanie rzetelnych informacji na temat składu jakościowego i ilościowego badanej próbki. Przy doborze odpowiedniej techniki pomiarowej należy kierować się m.in. takimi aspektami jak: charakter chemiczny oznaczanej substancji, rodzaj matrycy próbki, dokładność, czułość, precyzja oraz selektywność oznaczenia. W przypadku technik mikroekstrakcyjnych parametrem determinującym rodzaj zastosowanej techniki pomiarowej jest w dużej mierze objętość fazy organicznej uzyskanej w wyniku ekstrakcji. Objętość ta waha się od kilku do kilkunastu mikrolitrów, dlatego też techniki mikroekstrakcyjne stosowane są zazwyczaj w połączeniu z takimi technikami instrumentalnymi, które wymagają niewielkiej objętości próbki do przeprowadzenia pomiaru.

Na rys. 2 przedstawiono techniki pomiarowe stosowane do oznaczania jonów metali po zatężaniu DLLME. Zdecydowana większość publikacji dotyczy połączenia DLLME z absorpcyjną spektrometrią atomową z atomizacją w płomieniu (F-AAS). Pomimo swej ogromnej popularności, F-AAS wymaga jednak rozcieńczania wzbogaconej fazy organicznej w celu przeprowadzenia pomiaru, co powoduje znaczne obniżenie otrzymanych współczynników wzbogacenia do wartości mieszczących się w zakresie 15-50. Procedury analityczne oparte na połączeniu DLLME i F-AAS zastosowano m.in. do oznaczania: Li [16], Al [17], Cr [18-21], Mn [22], Fe [23-25], Co [26-31], Ni [32-34], Cu [35-46], Zn [47,48], Zr [49], Rh [50-52], Pd [53-57], Ag [58-60], Cd [61-68], Au [69,70], Tl [71,72], Pb [73-78], Bi [79] oraz pierwiastków ziem rzadkich [80]. Kolejną techniką pomiarową stosowaną w połączeniu z DLLME jest ET-AAS. Technika ta dedykowana jest do analizy próbek o niewielkiej objętości, dzięki czemu uzyskuje się współczynniki wzbogacenia rzędu 50-400. Przy użyciu DLLME/ET-AAS oznaczano szereg jonów metali, m.in.: V [81,82], Cr [83-87], Mn [88], Co [89-91], Ni [92-94], Cu [95-97], Zn [98,99], As [100-104], Se [105,106], Mo [107,108], Pd [109-113], Ag [114-119], Cd [120-122], Sb [123], Te [124], Au [125-127], Hg [128], Pb [129-132] oraz Tl [133].



Rys. 2. Techniki spektroskopowe stosowane w oznaczaniu jonów metali techniką DLLME. Wyjaśnienie akronimów przedstawiono na stronach 7-8.

W ciągu ostatnich trzech lat wzrosła także liczba publikacji dotyczących połączeń DLLME ze spektrofotometrią UV-Vis oraz technikami jej pokrewnymi [134]. Zbyt duża objętość kuwet pomiarowych stosowanych standardowo w tych technikach (ok. 3500 µL), wymaga rozcieńczania ekstraktu przed oznaczeniem. Dzięki wprowadzeniu mikro-kuwet o pojemności 50 µL można uzyskać współczynniki wzbogacenia rzędu 160 [135].

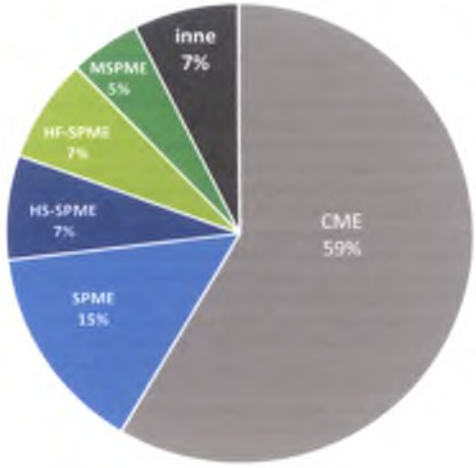
DLLME stosowana jest także w połączeniu z technikami dedykowanymi pomiarowi próbek stałych takich jak: laserowo indukowana spektroskopia emisyjna (LIBS) [136], spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie z ablacją laserową (LA-ICP-MS) [137] lub rentgenowska spektrometria fluorescencyjna (XRF) [138].

4.2. MIKROEKSTRAKCJA DO FAZY STAŁEJ

Mikroekstrakcja do fazy stałej znalazła gównie zastosowanie do zatężania i izolowania związków organicznych. W mikroekstrakcji do fazy stacjonarnej (SPME) anality ekstrahowane są do cienkiej warstwy adsorbentu, pokrywającej włókno szklane, kwarcowe lub stalowe. Najczęściej stosowanymi fazami stacjonarnymi w SPME są polisiloksany, np. polidimetylosiloksan lub jego mieszanina z diwinylobenzenem. Polisiloksany są stabilne chemicznie i termicznie a ich struktura pozwala na łatwą sorpcję analitów. Ponadto można łatwo sterować selektywnością procesu adsorpcji poprzez zakotwiczanie na powierzchni tych polimerów różnych grup funkcyjnych. Adsorbent może być zanurzony bezpośrednio w analizowanym roztworze lub umieszczony w przestrzeni nad nim (mikroekstrakcja do fazy stacjonarnej z fazy nadpowierzchniowej, HS-SPME) [139-141]. Po ekstrakcji anality desorbowane są termicznie lub z zastosowaniem rozpuszczalników organicznych bezpośrednio do odpowiedniego instrumentu pomiarowego (np. do dozownika chromatografu gazowego). Termiczna desorpcja prowadzona jest zazwyczaj w temperaturze 150-300°C ze względu na praktycznie całkowity odzysk analitów. Nowe rozwiązania w zakresie stosowalności SPME obejmują m.in. automatyzację oraz zastosowanie nowych faz sorpcyjnych (np. ciecze jonowe, nanorurki węglowe, grafen, adsorbenty na osnowie krzemionkowej).

W dyspersyjnej ekstrakcji do mikro-fazy stałej (DMSPE) [142-144] stały adsorbent rozpraszany jest w analizowanej próbce ciekłej. Następnie próbka przepuszczana jest np. przez membranowe filtry nylonowe, po czym przeprowadza się termiczną desorpcję [145], elucję [146,147] lub bezpośrednio analizuje oznaczane substancje przy użyciu odpowiedniej techniki pomiarowej [148]. Klasyczne adsorbenty stosowane w ekstrakcji coraz częściej zastępowane są przez różnego rodzaju nanomateriały, które charakteryzują się dużą powierzchnią właściwą oraz pojemnością sorpcyjną. Nanomateriały umożliwiają szybką i ilościową sorpcję analitów, a także, co jest niezwykle istotne podczas analizowania próbek ciekłych, wysoki stopień dyspersji w roztworach. Nanocząstki, które znalazły zastosowanie w DMSPE, można podzielić na dwie główne grupy: oparte na węglu (np. fulereny, nanorurki węglowe, grafen) oraz nieorganiczne, do których zalicza się m.in. nanocząstki magnetyczne [149,150].

Liczne zalety mikroekstrakcji do fazy stałej sprawiają, że jest ona coraz częściej stosowana do zatężania śladowych ilości jonów metali ciężkich, a jej popularność stale wzrasta. Na rys. 3 przedstawiono typy mikroekstrakcji do fazy stałej stosowane w analizie nieorganicznej. Jedną z najbardziej popularnych technik SPME jest mikroekstrakcja kapilarna (CME), w której adsorbent o dużym powinowactwie do oznaczanej substancji pokrywa wewnętrzną ścianę kolumny kapilarnej. Jako adsorbenty w CME stosowane są m.in.: Al_2O_3 [151,152], ZrO_2 [153], TiO_2 [154], modyfikowana krzemionka [155-159], polimery [160] oraz jednościenne nanorurki węglowe [161]. Kolejną techniką, która znalazła zastosowanie w analizie nieorganicznej, jest membranowa mikroekstrakcja do fazy stacjonarnej (MSPME).

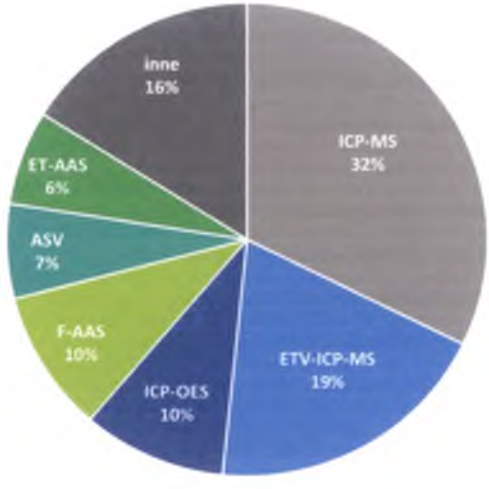


Rys. 3. Typy SPME stosowane w oznaczaniu śladowych ilości metali. Wyjaśnienie akronimów przedstawiono na stronach 7-8.

W MSPME stosuje się włókno ekstrakcyjne (np. szklane lub polipropylenowe) z naniesionym na jego powierzchni adsorbentem o charakterze polarnym, które odgrodzone jest hydrofobową membraną od analizowanego roztworu. Membrana ta umożliwia stosowanie adsorbentów rozpuszczalnych w wodzie, a także pozwala na regulowanie selektywności sorpcji poprzez zmianę stałej dielektrycznej adsorbentu. W zatężaniu śladowych ilości jonów metali zastosowano do tej pory włókna pokryte TiO_2 [162] oraz Al_2O_3 [163]. Innym wariantem SPME jest mikroekstrakcja poprzez membranę do fazy stałej (HF-SPME), w której adsorbent umieszczony jest wewnątrz mikroporowatego włókna ekstrakcyjnego. Opracowano procedury oznaczania śladowych ilości metali wykorzystujące włókna polipropylenowe wypełnione utlenionymi wielościennymi nanorurkami węglowymi [164] oraz mieszaniną tlenku grafenu i krzemionki [165].

Techniki pomiarowe stosowane w oznaczaniu jonów metali zatężanych metodami SPME. Spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie (ICP-MS) jest najczęściej stosowaną techniką w połączeniu z mikroekstrakcją do fazy stałej (rys. 4). Technika ta charakteryzuje się wysoką czułością, szerokim zakresem liniowości oraz niskimi granicami wykrywalności. Ponadto umożliwia oznaczanie zawartości wielu pierwiastków w jednej próbce oraz oznaczanie izotopów. Problemy związane ze stosowaniem techniki ICP-MS związane są głównie z występowaniem interferencji spektralnych oraz niekorzystnym wpływem matrycy próbki. Interferencje spektralne można wyeliminować np. dzięki zastosowaniu wysokorozdzielczej spektrometrii mas z jonizacją w plazmie sprzężonej indukcyjnie (HR-ICP-MS), natomiast wpływ matrycy może być zniwelowany poprzez stosowanie technik izolacji i zatężania analitów. ICP-MS w połączeniu z mikroekstrakcją do fazy stałej znalazła zastosowanie do oznaczania m.in.: Co, Ni, Cd [151,160], Cr, Cu, Cd, Pb [153], Mn [159], a także analizy specjacyjnej Cr i As [152] oraz As i Se [158] w połączeniu z CME. Ponadto opracowano procedury: (i) SPME/ICP-MS dla oznaczania Ti, Fe, Co, Ni, Cu, Ga, Cd, Sn oraz pierwiastków ziem rzadkich [166], (ii) HF-SPME/ICP-MS dla Mn, Co, Ni, Cu, Cd i Pb [165] oraz Fe, Cu, Zn, Cd i Pb [167], a także (iii) M-SPME/ICP-MS dla V, Co, Ni i Cd [162].

Kolejną techniką stosowaną do oznaczania śladowych ilości metali jest atomowa spektrometria optyczna ze wzbudzeniem w plazmie sprzężonej indukcyjnie (ICP-OES). Technika ta charakteryzuje się wysoką selektywnością, czułością oraz powtarzalnością. Wykazuje także lepszą tolerancję na obecność odczynników organicznych i próbek zasolonych niż ICP-MS. ICP-OES znalazła zastosowanie do jednoczesnego oznaczania jonów Cu, Hg oraz Pb techniką CME [155] oraz jonów Cu, Mn i Ni przy użyciu SPME ze wspomaganiem elektrochemicznym [163]. Głównym ograniczeniem podczas stosowania ekstrakcji do fazy stałej w połączeniu z ICP-OES jest dobór odpowiedniego adsorbentu, który umożliwiałby ilościową sorpcję oraz elucję analitów. Rozwiązaniem tego problemu może być dozowanie zawiesiny do komory spektrometru, co w konsekwencji skraca czas przygotowania próbki łącząc w jeden etap rozkład matrycy próbki, atomizację oraz wzbudzenie oznaczanych pierwiastków. Rozwiązanie to zastosowano do jednoczesnego oznaczania Be, V, Cr, Mn, Co, Cu, As, Se, Mo, Cd, Sb, Hg i Pb w próbkach biologicznych oraz środowiskowych [168]. Techniką spektroskopową, która pozwala na bezpośrednią analizę próbek ciekłych o objętości kilku mikrolitrów oraz próbek stałych o masie kilku miligramów jest spektrometria mas z jonizacją w plazmie sprzężonej indukcyjnie z elektrotermicznym odparowaniem próbki (ETV-ICP-MS). Zaproponowano procedury ETV-ICP-MS z (i) MSPME do oznaczania Cd, Hg i Pb [169], (ii) HS-SPME do zatężania Se, Te i Bi [140] a także (iii) CME dla V, Cr, Cu [154], Al [156] oraz As [157]. Pomiar małych objętości próbki umożliwia także ET-AAS [170,171]. Stosowanie technik wstępnego zatężania i izolacji analitów konieczne jest także w przypadku F-AAS. Wstępne przygotowanie próbki to analizy polepsza niezbyt wysoką czułość tej techniki pomiarowej. Przy użyciu F-AAS oznaczano m.in. Pd [172] oraz Cd i Pb [173].



Rys. 4. Techniki spektroskopowe stosowane w mikroekstrakcji do fazy stałej. Wyjaśnienie akronimów przedstawiono na stronach 7-8.

4.3. RENTGENOWSKA SPEKTROMETRIA FLUORESCENCYJNA W OZNACZANIU JONÓW METALI CIĘŻKICH

Technikami spektroskopowymi najczęściej wybieranymi do oznaczania śladowych ilości pierwiastków w próbkach ciekłych są: F-AAS, ET-AAS, ICP-OES oraz ICP-MS. Pomimo ogromnej czułości i selektywności tych technik, przygotowanie próbki do bezpośredniego pomiaru (poprzez wzbogacanie i izolację analitu z matrycy próbki) jest często niezbędnym etapem pozwalającym na uzyskanie precyzyjnych i dokładnych wyników.

Rentgenowska spektrometria fluorescencyjna (XRF) jest powszechnie znaną techniką analityczną, która znalazła szerokie zastosowanie w analizie jakościowej i ilościowej, m.in. ze względu na możliwość oznaczania wielu pierwiastków w jednej próbce oraz możliwość analizowania próbek stałych bez konieczności wcześniejszego przeprowadzenia ich do roztworu. Bezpośrednia analiza próbek ciekłych technikami rentgenowskiej spektrometrii fluorescencyjnej z dyspersją energii (EDXRF) i dyspersją długości fal (WDXRF) niesie ze sobą wiele utrudnień technicznych, które skutkują znacznymi błędami w otrzymanych wynikach. W XRF próbki ciekłe analizowane są bezpośrednio przy użyciu kaset pomiarowych z cienką warstwą folii (Mylar, polietylen, Kapton). Typowe granice wykrywalności przy czasie pomiaru wynoszącym około 5-30 minut są rzędu mg L^{-1} co nie jest wystarczające w przypadku oznaczania śladowych ilości pierwiastków w próbkach środowiskowych. Ponadto próbki ciekłe intensywnie rozpraszają promieniowanie rentgenowskie, co skutkuje wysokim tłem i w konsekwencji niskim stosunkiem sygnału do tła. Redukcja wysokiego tła może być osiągnięta m.in. przez polaryzację promieniowania rentgenowskiego lub zastosowanie rentgenowskiej spektrometrii fluorescencyjnej z całkowitym odbiciem promieniowania (TXRF) [174-177]. W TXRF wiązka promieniowania pierwotnego pada na reflektor pod bardzo małym kątem (~0.1°) dzięki czemu promieniowanie ulega całkowitemu odbiciu. Ze względu na to, że promieniowanie ulega rozproszeniu jedynie w próbce naniesionej na reflektor a nie w samym reflektorze, uzyskuje się poprawę stosunku sygnału do tła. TXRF daje możliwość szybkiego i prostego analizowania próbek ciekłych i zawiesin przy relatywnie niskich kosztach.

Analiza próbek ciekłych technikami XRF w „klasycznej" geometrii pomiarowej wymaga stosowania różnego rodzaju technik izolacji i zatężania analitów. Największą popularnością cieszą się te techniki wzbogacania, które pozwalają uzyskać próbki w postaci cienkiej warstwy. Możliwe jest wówczas zaniedbanie efektów matrycy, a uzyskane tło jest bardzo niskie. Do zatężania śladowych ilości analitów przed pomiarem XRF stosowano m.in.: odparowanie, elektroosadzanie, strącanie, ekstrakcję ciecz-ciecz, ekstrakcję ciecz-ciało stałe [178]. Ze względu jednak na ich czasochłonność oraz często konieczność stosowania dużych ilości odczynników chemicznych, klasyczne techniki wzbogacania zostały wyparte przez techniki mikroekstrakcyjne typu ciecz-ciecz i ciecz-ciało stałe. Połączenie technik mikroekstrakcyjnych z analizą XRF pozwala wyeliminować etap elucji analitów z powierzchni stałych adsorbentów, a także nie wymaga rozcieńczania wzbogaconej fazy organicznej przed pomiarem, dzięki czemu możliwe jest uzyskanie wysokich współczynników wzbogacenia oraz niskich granic wykrywalności. Ze względu na możliwość analizy próbek o bardzo małych

objętościach, również spektrometria TXRF znalazła zastosowanie do detekcji pierwiastków zatężanych techniką mikroekstrakcji do fazy ciekłej [138,179]. Pierwsze prace, jakie ukazały się w tematyce zastosowania mikroekstrakcji ciecz-ciecz do zatężania śladowych ilości jonów metali przed oznaczaniem techniką EDXRF, są przedmiotem niniejszej pracy doktorskiej **[D1, D2]**.

Ekstrakcja do fazy stałej w połączeniu z rentgenowską spektrometrią fluorescencyjną cieszy się w ostatnich latach coraz większą popularnością. Tendencja do stosowania ekstrakcji do fazy stałej związana jest z syntezą nowych materiałów sorpcyjnych oraz możliwością łatwego przygotowania jednorodnych próbek w postaci cienkiej warstwy [180]. Do tej pory zastosowano adsorbenty takie jak żywice syntetyczne [181,182], modyfikowana krzemionka [183], pianki poliueratanowe [184,185] oraz węgiel aktywny [186,187]. Nowymi materiałami, które znalazły zastosowanie w technikach ekstrakcyjnych i mikroekstrakcyjnych są nanomateriały węglowe [188-191]. Zastosowanie nanorurek węglowych [192-196], tlenku grafenu [197,198] i grafenu do oznaczania pierwiastków śladowych i ultraśladowych technikami rentgenowskiej fluorescencji jest obecnie przedmiotem badań prowadzonych w Zakładzie Chemii Analitycznej Instytutu Chemii Uniwersytetu Śląskiego [197-204] w tym również badań zrealizowanych w ramach niniejszej pracy doktorskiej **[D3-D5]**.

5. CEL PRACY

Celem badań przeprowadzonych w ramach niniejszej rozprawy doktorskiej było opracowanie metod zatężania i oznaczania pierwiastków śladowych opartych na dyspersyjnej mikroekstrakcji ciecz-ciecz (DLLME) oraz dyspersyjnej ekstrakcji do mikro-fazy stałej (DMSPE) oraz technikach rentgenowskiej spektrometrii fluorescencyjnej (EDXRF i TXRF). Badania nad nowymi procedurami analitycznymi obejmowały:

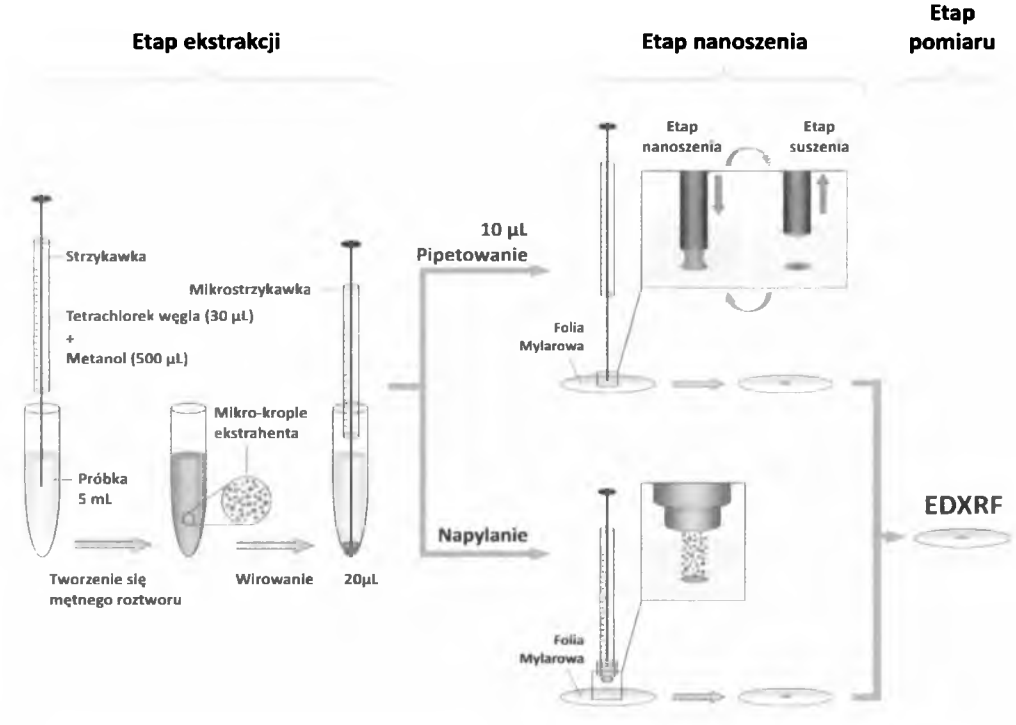
- opracowanie warunków prowadzenia ekstrakcji DLLME i DMSPE w celu uzyskania wysokich odzysków oznaczanych pierwiastków,
- opracowanie metod nanoszenia fazy wzbogaconej w oznaczane pierwiastki na podłoże odpowiednie do pomiaru EDXRF lub TXRF,
- dobór warunków pomiarowych w celu uzyskania optymalnej czułości metod i możliwie niskich granic wykrywalności,
- określenie podstawowych parametrów walidacyjnych.

6. OPIS PUBLIKACJI

6.1. DYSPERSYJNA MIKROEKSTRAKCJA TYPU CIECZ-CIECZ Z POMIAREM EDXRF

W pierwszym etapie prowadzonych badań opracowano procedury analityczne, w których po raz pierwszy wykorzystano dyspersyjną mikroekstrakcję typu ciecz-ciecz (DLLME) oraz spektrometrię EDXRF do oznaczania śladowych ilości jonów metali. Wyniki tej części badań zostały opublikowane w artykułach **[D1]** i **[D2]**.

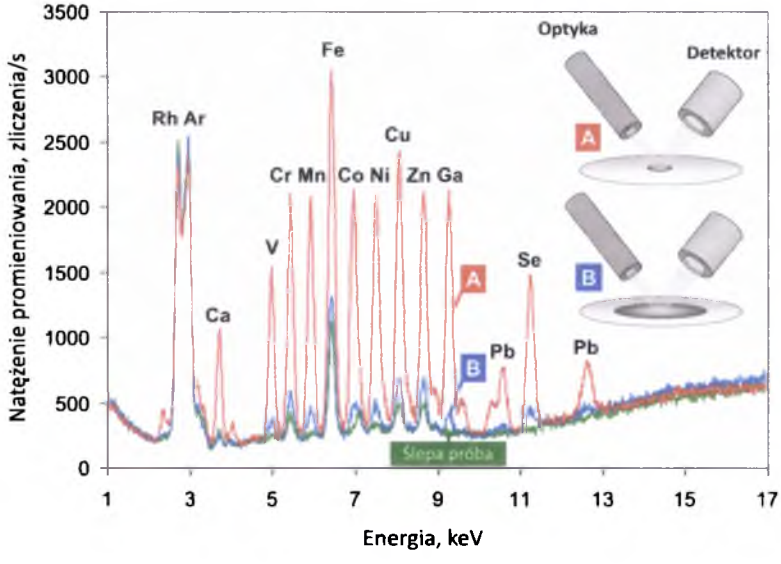
W pracy **[D1]** przebadano możliwość jednoczesnego oznaczania jonów Fe(III), Co(II), Zn(II), Se(IV), Ga(III) oraz Pb(II) przy użyciu APDC jako odczynnika chelatującego, tetrachlorku węgla jako rozpuszczalnika ekstrahującego oraz metanolu jako rozpuszczalnika rozpraszającego. Procedura przedstawiona w artykule **[D2]** pozwala na jednoczesne wzbogacanie jonów Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Se(IV) oraz Pb(II) z wykorzystaniem tetrachlorku węgla, metanolu oraz Na-DDTC w charakterze odczynnika chelatującego. Procedurę DLLME/EDXRF można podzielić na trzy podstawowe etapy: (i) wstrzyknięcie mieszaniny ekstrahent/dyspergator (CCl_4+CH_3OH) do analizowanej próbki i odwirowanie mętnego roztworu, (ii) nanoszenie fazy organicznej na podłoże oraz (iii) pomiar techniką EDXRF. Stosunek objętości rozpuszczalnika ekstrahującego do rozpraszającego został dobrany tak, by możliwe było utworzenie stabilnych mikro-kropel ekstrahenta rozproszonych w analizowanym roztworze [125,199]. Nanoszenie ekstraktu na podłoże (filtr Millipore) eliminuje konieczność jego rozcieńczania, dzięki czemu otrzymane wartości współczynników wzbogacenia są bardzo wysokie i wynoszą ok. 250. Schemat opracowanej procedury DLLME/EDXRF przedstawiono na rys 5.



Rys. 5. Schemat procedury DLLME/EDXRF.

W opracowanej procedurze DLLME/EDXRF dopasowanie średnicy otrzymanej próbki do rozmiarów mikrowiązki promieniowania rentgenowskiego ma decydujący wpływ na natężenie promieniowania emitowanego przez pierwiastki. Na rys. 6 przedstawiono widma EDXRF próbek o średnicach 1 i 5 mm (oznaczonych odpowiednio A i B), zawierających jednakową ilość oznaczanych pierwiastków (500 ng). Próbki wzbudzano wiązką pierwotnego promieniowania rentgenowskiego o średnicy 0,9 mm. W przypadku próbki B jedynie niewielka ilość wyekstrahowanych pierwiastków zostaje wzbudzona, co znacząco wpływa na zmniejszenie intensywności emitowanego promieniowania fluorescencyjnego. Otrzymane wyniki pokazują, że gdy średnica próbki jest dopasowana do rozmiarów mikrowiązki obserwuje się wysoką intensywność promieniowania i bardzo dobry stosunek piku do tła.
Dla uzyskania próbek w postaci cienkiej warstwy i najlepszego dopasowania średnicy otrzymanej próbki do rozmiarów wiązki promieniowania rentgenowskiego, a w związku z tym najlepszego stosunku sygnału do tła, opracowane zostały trzy sposoby nanoszenia wzbogaconej fazy organicznej na podłoże. Próbki o średnicy kilku mm otrzymano poprzez:

- pipetowanie, czyli nanoszenie mikro-objętości fazy organicznej na filtry o średnicy ok. 1,5 mm i każdorazowe odparowaniu rozpuszczalnika (przy użyciu promiennika IR),
- nanoszenie ekstraktu w bardzo małych porcjach (ok. 1 µL) na filtry o standardowych średnicach (np. 25 albo 47 mm) i natychmiastowe suszenie,
- napylanie otrzymanego ekstraktu w strumieniu gazu; próbki napylano wykorzystując aparaturę stosowaną w chromatografii cienkowarstwowej (Applicator As 30, Desaga HPTLC ze strzykawką dozującą o objętości 10 µL). Jako podłoże stosowano zarówno folię Mylarową jak i filtry Millipore.



Rys. 6. Pomiar próbki zawierającej 500 ng oznaczanych metali techniką EDXRF (A- próbka o średnicy 1 mm, B- próbka o średnicy 5 mm).

Warto podkreślić, że nie tylko średnica otrzymanej próbki ma wpływ na intensywność promieniowania emitowanego przez pierwiastki. Ważne jest również zjawisko samoabsorpcji promieniowania rentgenowskiego, związane w dużej mierze z grubością analizowanej próbki. Uzyskana grubość próbki zależy m.in. od sposobu dodawania odczynnika kompleksującego do badanego roztworu. Możliwe są dwa przypadki:

- odczynnik dodawany jest do próbki przed ekstrakcją (np. APDC, Na-DDTC); w kolejnym etapie utworzone kompleksy metali ekstrahowane są do fazy organicznej, zatem zawartość odczynnika kompleksującego w napylanej próbce jest proporcjonalna do stężenia analitów;
- odczynnik dodawany jest bezpośrednio do fazy organicznej (np. ditizon); ilość odczynnika kompleksującego w napylanym na filtr roztworze jest wysoka, co prowadzi do zwiększenia grubości próbki i w konsekwencji uwydatnienia się efektów związanych z absorpcją promieniowania.

Zależność pomiędzy natężeniem promieniowania emitowanym przez pierwiastki i średnicą wysuszonej próbki szczegółowo opisano w pracy **[D1]**. Jeżeli odczynnik kompleksujący (APDC lub Na-DDTC) dodawany jest do analizowanego roztworu przed ekstrakcją, wówczas praktycznie nie są obserwowane efekty związane z samoabsorpcją, a zwiększanie średnicy analizowanej próbki powoduje obniżenie intensywności promieniowania rentgenowskiego, ponieważ tylko część wyekstrahowanych pierwiastków ulega wzbudzeniu. Innymi słowy natężenie promieniowania maleje wraz ze zmniejszaniem się masy powierzchniowej próbki (rys. 4 w **[D1]**). Jeśli odczynnik kompleksujący (ditizon) znajduje się w fazie organicznej, efekty związane z absorpcją promieniowania uwidaczniają się najbardziej dla próbek o najmniejszej średnicy i pierwiastków emitujących promieniowanie długofalowe (np. Mn, Fe, Co). Po osiągnięciu wartości maksymalnej natężenie promieniowania maleje, ponieważ wraz z dalszym zwiększaniem średnicy próbki zmniejsza się liczba atomów wzbudzanych przez wiązkę promieniowania pierwotnego padającego na próbkę.

W pracy **[D2]** porównano właściwości APDC oraz Na-DDTC jako nieselektywnych odczynników kompleksujących. Najważniejszym parametrem wpływającym na chelatowanie metali jest pH analizowanego roztworu. Wpływ pH na odzysk pierwiastków został sprawdzony dla zakresu od 1 do 11. Wyniki dla APDC i Na-DDTC przedstawiono na rys. 1 zamieszczonym w publikacji **[D2]**. W przypadku Na-DDTC prowadzenie ekstrakcji przy pH 5 umożliwia jednoczesne wzbogacanie jonów Fe(III), Co(II), Ni(II), Cu(II), Zn(II) i Pb(II), czyli sześciu z siedmiu oznaczanych pierwiastków (powyżej pH 4 wydajność ekstrakcji Se zmniejsza się gwałtownie). Należy podkreślić, że prowadzenie ekstrakcji przy pH 2 umożliwia oddzielenie Se od pierwiastków, które w próbkach środowiskowych występują często w dużych stężeniach tj. od Fe oraz Zn. Prowadzenie ekstrakcji przy pH 3 z APDC jako odczynnikiem kompleksującym umożliwiło jednoczesne wzbogacanie jonów Co(II), Zn(II), Pb(II), Se(IV), Fe(III) oraz Ga(III).

W pracach **[D1]** i **[D2]** szczegółowo przebadano niepewności związane z każdym z poszczególnych etapów opracowanej procedury analitycznej tj. z etapem ekstrakcji, etapem nanoszenia fazy organicznej na podłoże oraz z etapem pomiaru. Na niepewność

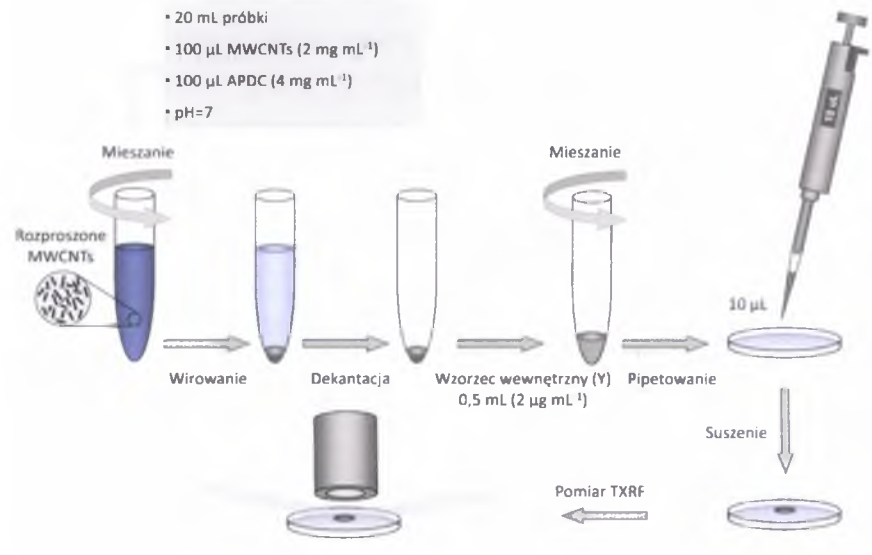
pomiaru przy użyciu EDXRF składają się błędy związane z pozycjonowaniem próbki, niestabilnością aparatury oraz statystyką zliczeń fotonów. Otrzymane wyniki pokazały, że największy wpływ na całkowitą niepewność procedury DLLME/EDXRF ma etap ekstrakcji pierwiastków (wartości wahają się od 3,0 do 13,1% dla APDC oraz od 2,3 do 11,5% dla Na-DDTC). Najmniejszy wpływ na precyzję miały natomiast błędy wynikające z niestabilności przyrządu pomiarowego i statystyki zliczeń fotonów (poniżej 1,5% dla Fe, Co, Ni, Cu, Zn, Ga i Se oraz około 3,0% dla Pb).

Wyznaczono również zakres liniowości procedury DLLME/EDXRF (rys. 6 w pracy **[D1]** oraz rys. S1 w pracy **[D2]**). Liniową zależność pomiędzy intensywnością promieniowania a zawartością oznaczanych jonów metali obserwowano do stężenia wynoszącego 0,4 μg mL^{-1}. Po przekroczeniu tej wartości zaczynały wytrącać się kompleksy oznaczanych pierwiastków w postaci trudno rozpuszczalnych osadów. Ze względu na bardzo częste występowanie względnie wysokich stężeń jonów Fe(III) i Zn(II) w próbkach środowiskowych, sprawdzono także zakres liniowości dla roztworów wzorcowych zawierających dziesięć razy wyższe stężenie tych pierwiastków. Liniową zależność pomiędzy intensywnością promieniowania a stężeniem pierwiastka obserwowano do stężenia wynoszącego 0,2 μg mL^{-1}. Węższy niż wcześniej zakres linowości może być wytłumaczony współstrącaniem się pierwiastków razem z kompleksami Zn/Fe-APDC lub Zn/Fe-DDTC. Opracowane procedury umożliwiają uzyskanie granic wykrywalności na poziomie 1,6-4,1 ng mL^{-1} **[D1]** oraz 1,5-3,9 ng mL^{-1} **[D2]**. Warto zaznaczyć, że tak niskie granice wykrywalności są możliwe do uzyskania technikami XRF tylko przy dużych objętościach próbek tj. 100-1000 mL [178]. W pracach **[D1]** i **[D2]** dzięki wysokiemu współczynnikowi wzbogacenia, wynoszącemu ok. 250, oraz opracowaniu metody nanoszenia fazy organicznej na podłoże w postaci małej plamki dopasowanej do średnicy wiązki, uzyskano tak niskie granice wykrywalności dla próbek o objętości 5 mL. Ponadto otrzymane granice wykrywalności znajdują się poniżej maksymalnych dopuszczalnych poziomów zanieczyszczeń dla wód pitnych ustanowionych przez amerykańską Agencję Ochrony Środowiska (EPA) [200] i norm znajdujących się w Rozporządzeniu Ministra Zdrowia w sprawie naturalnych wód mineralnych, naturalnych wód źródlanych i wód stołowych [201].

Wyniki oznaczeń uzyskanych opracowaną procedurą DLLME/EDXRF porównano z rezultatami otrzymanymi technikami F-AAS (bezpośrednie oznaczanie jonów Fe i Zn) oraz ET-AAS (Co, Ga, Se oraz Pb zostały wcześniej oddzielone i zatężone przy użyciu klasycznej ekstrakcji do fazy stałej). Otrzymane wyniki pokazały, że względna różnica pomiędzy ET-AAS a EDXRF waha się od 6,4% dla Fe do 10,3% dla Zn w przypadku APDC oraz od 2,0% dla Pb do 15,4% dla Co w przypadku Na-DDTC (tabela 1 w pracy **[D1]** oraz tabela 2 w pracy **[D2]**).

6.2. EKSTRAKCJA DO MIKRO-FAZY STAŁEJ Z POMIAREM TXRF

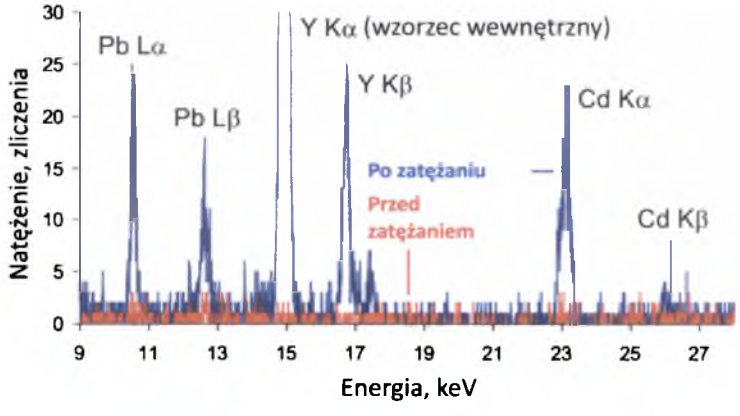
W pracy **[D3]** po raz pierwszy zastosowano wzbogacanie śladowych ilości jonów metali ciężkich techniką dyspersyjnej ekstrakcji do mikro-fazy stałej (DMSPE) z zastosowaniem utlenionych wielościennych nanorurek węglowych jako adsorbentem wraz z pomiarem techniką rentgenowskiej spektrometrii fluorescencyjnej z całkowitym odbiciem promieniowania (TXRF). W procedurze opisanej w publikacji **[D3]**, zawiesina nanorurek węglowych wstrzykiwana jest do analizowanego roztworu, co zapewnia duży stopień rozproszenia nanosorbentu i szybkie osiągnięcie stanu równowagi. Ponadto istniejące na powierzchni utlenionych nanorurek węglowych grupy funkcyjne (karboksylowe, hydroksylowe oraz karbonylowe) są odpowiedzialne za właściwości hydrofilowe tych nanomateriałów i dużo lepszą rozpraszalność w wodzie w porównaniu z nieutlenionymi nanorurkami węglowymi. Zastosowane podejście skraca czas przygotowania próbki, co czyni procedurę DMSPE/TXRF nieskomplikowanym narzędziem umożliwiającym oznaczanie zawartości wielu pierwiastków w jednej próbce. Ponadto w odróżnieniu od klasycznej ekstrakcji do fazy stałej, nie jest konieczne stosowanie kolumn wypełnionych warstwą adsorbentu, co rozwiązuje problemy związane ze stratami złoża wynikającymi z wydostawaniem się nanocząstek adsorbentu z wnętrza kolumny. Po odwirowaniu, do nanorurek węglowych znajdujących się na dnie probówki dodawano odpowiednią ilość wzorca wewnętrznego, po czym 10 µL zawiesiny nanoszono na reflektor kwarcowy i suszono z zastosowaniem promiennika IR. Opracowaną procedurę zastosowano do oznaczania jonów Cd(II) i Pb(II). Schemat procedury DMSPE/TXRF przedstawiono na rys. 7. W pracy **[D3]** dokonano doboru takich parametrów jak: pH analizowanego roztworu, objętość próbki, ilość adsorbentu, ilość odczynnika kompleksującego oraz czas prowadzenia adsorpcji.



Rys. 7. Schemat procedury DMSPE/TXRF.

Badania mające na celu określenie wpływu pH na odzysk jonów Cd(II) i Pb(II) przeprowadzono w obecności odczynnika kompleksującego (APDC) oraz przy jego braku (rys. 2 w pracy **[D3]**). Zastosowanie APDC pozwala na oznaczanie pierwiastków z wysokimi odzyskami dla zakresu pH od 2 do 12 dla jonów Cd(II) i od 4 do 9 dla jonów Pb(II). Otrzymane wyniki pokazały również, że obecność odczynnika kompleksującego wpływa na polepszenie precyzji procesu sorpcji. Do jednoczesnego oznaczania jonów Cd(II) i Pb(II) w próbkach wód wybrano pH 7. Innym parametrem, który znacząco wpływa na odzysk jonów Cd(II) i Pb(II) jest ilość stosowanego adsorbentu. Zadowalające odzyski otrzymano dla masy adsorbentu wahającej się pomiędzy 5 a 10 µg w jednym mililitrze analizowanego roztworu. Dalsze zwiększanie stężenia adsorbentu przyczynia się do spadku natężenia promieniowania charakterystycznego emitowanego przez Cd i Pb, co związane jest z powstawaniem próbek o większych grubościach (rys. 3.b w pracy **[D3]**).

Jednym z ważniejszych aspektów, na jaki zwrócono uwagę podczas opracowywania przedstawionej w publikacji **[D3]** procedury DMSPE/TXRF, było przygotowanie próbek w postaci cienkiej warstwy naniesionej na powierzchnię reflektora kwarcowego. Dla uzyskania optymalnych warunków całkowitego odbicia promieniowania rentgenowskiego, sprawdzono parametry takie jak: (i) objętość zawiesiny nanoszonej na reflektor, (ii) sposób suszenia próbki (wyciąg z pionowym przepływem laminarnym, promiennik IR, płyta grzejna) oraz (iii) czas prowadzenia pomiaru (rys. 4 w pracy **[D3]**). Uzyskane wyniki pokazują, że objętość nanoszonej na reflektor zawiesiny nanorurek węglowych ma nieznaczny wpływ na wartość natężenia promieniowania rentgenowskiego emitowanego przez Cd i Pb. Ponadto żaden z zaproponowanych sposobów suszenia nie ma wpływu na sygnał analityczny oznaczanych pierwiastków. Uzyskane wyniki pokazały również, że wraz ze wzrostem czasu pomiaru precyzja ulega poprawie. Dla czasu pomiaru wynoszącego 2000 s RSD waha się pomiędzy 5 a 10%. W kolejnych etapach badań na reflektor kwarcowy nanoszono 10 µL zawiesiny, którą suszono przy pomocy promiennika IR. Pomiar prowadzono przez 2000 s. Zalety zaproponowanej procedury DMSPE/TXRF potwierdzają widma TXRF przedstawione na rys. 8.



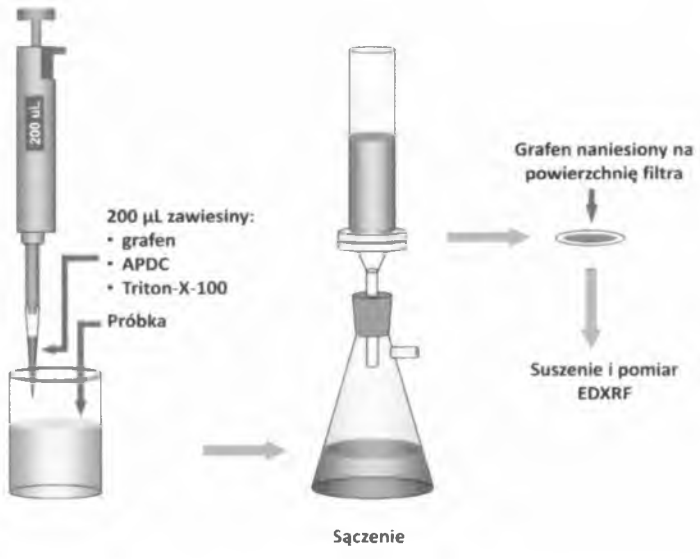
Rys. 8. Widma TXRF dwóch próbek, powstałych poprzez bezpośrednią analizę roztworu wzorcowego (czerwona linia) oraz roztworu wzorcowego poddanego zatężaniu techniką DMSPE (niebieska linia).

Widma te zostały zarejestrowane w trakcie pomiaru próbek zawierających jednakową ilość oznaczanych pierwiastków (0,2 ng), przy czym widmo zaznaczone kolorem czerwonym uzyskano dla próbki, która nie została poddana procedurze zatężania. Otrzymane wyniki pokazały, że zaobserwowanie sygnału analitycznego pochodzącego od oznaczanych pierwiastków jest możliwe tylko po zastosowaniu zatężania techniką DMSPE.

Zastosowanie DMSPE jako metody zatężania i TXRF jako techniki pomiarowej pozwoliło na uzyskanie niskich granic wykrywalności (1,0 ng mL^{-1} Cd(II) oraz 2,1 ng mL^{-1} Pb(II)). Otrzymane granice wykrywalności znajdują się poniżej maksymalnego poziomu zanieczyszczeń, który mogą zawierać wody pitne zgodnie z normami ustanowionymi przez EPA. Dla jonów Cd(II) jest to 5 ng mL^{-1} [202] a dla jonów Pb(II) 15 ng mL^{-1} [203]. Otrzymane granice wykrywalności znajdują się także poniżej dopuszczalnych stężeń ustanowionych w Rozporządzeniu Ministra Zdrowia w sprawie naturalnych wód mineralnych, naturalnych wód źródlanych i wód stołowych (3 ng mL^{-1} i 10 ng mL^{-1}, odpowiednio dla Cd i Pb) [201]. Uzyskanie tak niskich granic wykrywalności było możliwe dzięki wysokiemu współczynnikowi wzbogacenia wynoszącemu 40. Warto także podkreślić, że opracowana procedura DMSPE/TXRF pozwala na jednoczesne oznaczanie jonów Cd(II) i Pb(II) w próbkach wód o wysokim zasoleniu (woda morska) i bogatej matrycy organicznej (ścieki komunalne) (tabela 4 w pracy **[D3]**). Rzetelność procedury DMSPE/TXRF sprawdzono analizując próbki wody zawierające dodatek 20 i 50 ng mL^{-1} oznaczanych jonów. Odzyski dla przebadanych próbek wody wynosiły średnio 106 i 104%, odpowiednio dla Cd i Pb.

6.3. EKSTRAKCJA DO MIKRO-FAZY STAŁEJ Z POMIAREM EDXRF

Celem kolejnej części prowadzonych badań było zastosowanie grafenu jako efektywnego adsorbentu do jednoczesnego zatężania i oznaczania jonów Co(II), Ni(II), Cu(II) i Pb(II) **[D4]** oraz do analizy specjacyjnej selenu **[D5]**. Grafen zbudowany jest z atomów węgla o hybrydyzacji sp^2, tworzących przypominające plastry miodu, płaskie i sześciokątne warstwy. W odróżnieniu od nanorurek węglowych zastosowanych w charakterze adsorbentu w pracy **[D3]**, adsorpcja analitów może zachodzić z obydwu stron grafenu, a nie tak jak w przypadku nanorurek węglowych jedynie na zewnętrznych ścianach. Umożliwia to stosowanie dużo mniejszych ilości sorbentu (4 µg adsorbentu na 1 mL analizowanego roztworu) niż miało to miejsce w przypadku nanorurek węglowych (10 µg adsorbentu na 1 mL analizowanego roztworu). W opracowanej procedurze do analizowanej ciekłej próbki wstrzykiwana jest mieszanina składająca się z adsorbentu (1 mg mL^{-1} grafenu), odczynnika kompleksującego (4 mg mL^{-1} APDC) oraz środka powierzchniowo czynnego (0,5 mg mL^{-1} Tritonu-X-100). Szybkie nastrzyknięcie mieszaniny gwarantuje wysoki stopień rozproszenia grafenu w analizowanym roztworze, dzięki czemu równowaga procesu ustala się niemalże natychmiast i próbka może być przesączona bez konieczności wcześniejszego mieszania lub poddawania działaniu ultradźwięków. W kolejnym etapie próbki suszono przy użyciu promiennika IR. Schemat opracowanej procedury przedstawiono na rys. 9. Ze względu na właściwości hydrofobowe grafenu do otrzymania jego stabilnej zawiesiny konieczne było wprowadzenie niejonowego środka powierzchniowo czynnego. Przebadano zatem wpływ ilości Tritonu-X-100 na precyzję oznaczeń śladowych ilości jonów Co(II), Ni(II), Cu(II) i Pb(II).



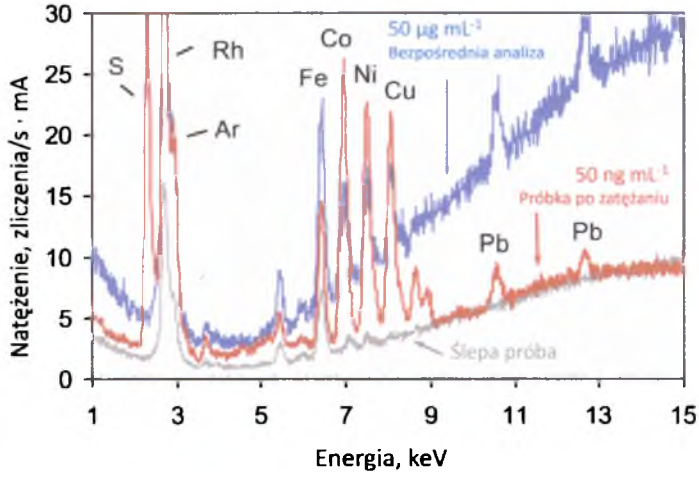
Rys. 9. Schemat procedury DMSPE/EDXRF.

Jednym z najważniejszych parametrów, mających bezpośredni wpływ na tworzenie kompleksów APDC z jonami metali i ich adsorpcję na powierzchni grafenu, jest pH analizowanego roztworu. Wpływ pH na odzysk pierwiastków sprawdzono dla zakresu 1-10, stosując cztery różne kombinacje odczynników (rys. 1 w pracy [D4]):

- grafen,
- grafen i APDC,
- grafen i Triton-X-100,
- grafen, APDC i Triton-X-100.

Na podstawie uzyskanych wyników do dalszych badań wybrano mieszaninę grafen/APDC/Triton-X-100, przy pH=5, co pozwoliło na jednoczesne oznaczanie jonów Co(II), Ni(II), Cu(II) i Pb(II) z odzyskiem bliskimi 100%.

Warto zaznaczyć, że procedura DMSPE/EDXRF pozwala na uzyskanie bardzo wysokich wartości współczynników wzbogacenia (wahających się od 400 do 2500), dzięki czemu uzyskane granice wykrywalności są bardzo niskie i wynoszą 0,08, 0,07, 0,08 i 0,20 ng mL^{-1}, odpowiednio dla jonów Co(II), Ni(II), Cu(II) oraz Pb(II). Na rys. 10 porównano widmo EDXRF powstałe w wyniku bezpośredniej analizy roztworu wzorcowego z widmem próbki stałej uzyskanej po zatężaniu techniką DMSPE/EDXRF. W widmie wyróżnionym kolorem czerwonym (uzyskanym przez zatężanie roztworu zawierającego 50 ng mL^{-1} oznaczanych pierwiastków) można zaobserwować znacznie lepszy stosunek sygnału do tła niż w widmie oznaczonym kolorem niebieskim, uzyskanym przez bezpośredni pomiar roztworu wzorcowego o stężeniu 50 µg mL^{-1}.

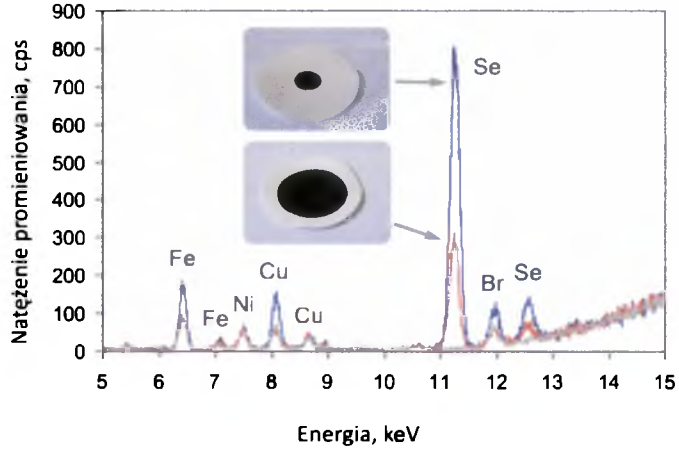


Rys. 10. Porównanie widm EDXRF pochodzących od próbki zawierającej 50 µg mL^{-1} oznaczanych pierwiastków (niebieska linia), próbki o stężeniu pierwiastków wynoszącym 50 ng mL^{-1} zatężanej przy użyciu procedury DMSPE/EDXRF (czerwona linia) oraz próbki ślepej (szara linia).

Kontynuacją badań dotyczących zastosowania grafenu jako adsorbentu w DMSPE/EDXRF było opracowanie procedury analitycznej umożliwiającej analizę specjacyjną Se. Wyniki tej części badań opublikowano w pracy **[D5]**. Jako odczynnik chelatujący zastosowano APDC, który tworzy kompleks jedynie z Se(IV). Stężenie Se(VI) obliczane było na podstawie różnicy pomiędzy całkowitą zawartością selenu po przeprowadzeniu etapu redukcji a stężeniem Se(IV).

Dla sprawdzenia, które z parametrów zatężania są statystycznie istotne, a zatem które z nich mają wpływ na odzysk Se, zastosowano centralny plan kompozycyjny z parametrem α=1 (FCCCD). Pozwoliło to na uzyskanie jak największej ilości informacji dotyczących procesu sorpcji (optymalnej wartości pH, ilości środka powierzchniowo czynnego, odczynnika kompleksującego oraz objętości analizowanego roztworu), przy jak najmniejszej ilości przeprowadzonych eksperymentów. Zakres przy którym badano pH ustalono pomiędzy 1 a 5, na podstawie informacji zawartych w publikacjach poświęconych specjacji Se z wykorzystaniem APDC jako odczynnika kompleksującego. Wartości dla pozostałych zmiennych ustalono na podstawie wstępnie wykonanych eksperymentów (stężenie APDC (0,4-2,0 mg mL^{-1}), stężenie Tritonu-X-100 (0,1-0,5 mg mL^{-1}) oraz objętość próbki (50-100 mL). Uzyskane w ten sposób wyniki pokazały, że jedynym parametrem mającym wpływ na odzysk Se jest pH (najgorsze odzyski dla pH 1, 3 i 5 wynosiły odpowiednio 96,1%, 88,3% oraz 18,1%). W związku z tym zastosowano FCCCD z trzema punktami centralnymi, w którym wartość pH ustalono na 1, natomiast wartości pozostałych zmiennych pozostały na tych samych co wcześniej poziomach. Uzyskane w ten sposób odzyski były podobne dla wszystkich przeprowadzonych eksperymentów i wynosiły od 97,0 do 103,6%. Można wnioskować, że żaden z trzech przebadanych parametrów w badanym zakresie (stężenie Tritonu-X-100, stężenie APDC oraz objętość próbki) nie ma wpływu na odzysk Se(IV).

W procedurze DMSPE/EDXRF średnica otrzymanej próbki znacząco wpływa na uzyskiwane wyniki. Powinna być ona dostatecznie mała, jeśli prowadzi się pomiary przy zastosowaniu wiązki promieniowania rentgenowskiego o niewielkich rozmiarach. Przedstawione na rys. 11 widma EDXRF dwóch próbek o średnicach 5 i 22 mm naniesionych na filtr Millipore i wzbudzonych przy użyciu wiązki promieniowania rentgenowskiego o średnicy ok. 5,9 mm, zawierają jednakową ilość Se (500 ng). Jeśli średnica próbki jest dopasowana do rozmiarów wiązki promieniowania pierwotnego obserwuje się wysoką intensywność promieniowania fluorescencyjnego i bardzo dobry stosunek piku do tła. Przedstawiona w pracy **[D5]** procedura DMSPE/EDXRF pozwala na uzyskanie wysokiego współczynnika wzbogacenia (ok. 1013), co przekłada się bezpośrednio na obniżenie otrzymanej granicy wykrywalności do wartości 0,032 ng mL^{-1}. Procedurę DMSPE/EDXRF zastosowano do oznaczania śladowych ilości jonów Se w próbkach wód (wodociąg, jezioro, morska) oraz do analizy specjacyjnej Se w próbkach wody mineralnej (tabele 3 i 4 w pracy **[D5]**). Otrzymane wyniki pokazują, że zastosowana w badaniach technika zatężania jest efektywna i dodatkowo odporna na wysokie stężenie jonów przeszkadzających (próbki wody morskiej), co znajduje swoje odzwierciedlenie w wysokich odzyskach (ok. 100%). Rzetelność procedury DMSPE/EDXRF sprawdzono analizując próbki materiałów odniesienia z certyfikowaną zawartością selenu (TORT-2, ERM®-BB186). Wyniki przedstawione w tabeli 5 (praca **[D5]**) pokazują, że opracowana procedura analityczna może również znaleźć zastosowanie do oznaczania śladowych ilości jonów selenu w próbkach biologicznych.



Rys. 11. Porównanie widm EDXRF uzyskanych dla próbek o średnicach 5 mm (niebieska linia) oraz 22 mm (czerwona linia), wzbudzonych wiązką pierwotnego promieniowania rentgenowskiego o średnicy 5,9 mm.

7. PODSUMOWANIE I WNIOSKI KOŃCOWE

Opracowano metody oznaczania pierwiastków na poziomie śladowym z zastosowaniem rentgenowskiej spektrometrii fluorescencyjnej (EDXRF i TXRF) oraz technik mikroekstrakcyjnych typu ciecz-ciecz (DLLME) i ciecz-ciało stałe (DMSPE). Podstawowe parametry walidacyjne opracowanych procedur przedstawiono w Tabeli 1.

Tabela 1. Porównanie metod zatężania będących podstawą rozprawy doktorskiej.

Pierwiastek	Technika zatężania	Technika pomiarowa	Objętość próbki (mL)	Współczynnik wzbogacenia	Granica wykrywalności (ng mL^{-1})	RSD (%)	Rodzaj analizowanej próbki	Lit.
Fe, Co, Zn, Ga, Se, Pb	DLLME	EDXRF	5	250	1,6 - 4,1	7,5 - 14,7	woda (rzeczna)	[D1]
Fe, Co, Ni, Cu, Zn, Se, Pb	DLLME	EDXRF	5	250	1,5 - 3,9	6,4 - 12,8	woda (rzeczna)	[D2]
Cd, Pb	DMSPE	TXRF	20	40	1,0 - 2,1	6,0 - 10,5	woda (morska, rzeczna) ścieki komunalne	[D3]
Co, Ni, Cu, Pb	DMSPE	EDXRF	50	418 - 2553	0,07 - 0,20	2,6 - 3,4	woda (wodociąg, jezioro, mineralna, morska)	[D4]
Se(IV), Se(VI)	DMSPE	EDXRF	75	1013	0,032	5,1	woda (wodociąg, jezioro, morska) próbki biologiczne	[D5]

- Połączenie technik mikroekstrakcyjnych z pomiarem EDXRF lub TXRF wydaje się obiecującym narzędziem umożliwiającym oznaczanie zawartości wielu pierwiastków w próbce. Wysokie wartości współczynników wzbogacenia oraz uzyskane próbki cienkie o średnicy dopasowanej do rozmiarów wiązki promieniowania rentgenowskiego prowadzą do granic wykrywalności od 0,03 do 4 ng mL^{-1}. Tak niskie granice wykrywalności są możliwe do uzyskania z zastosowaniem „klasycznych" metod zatężania tylko wówczas, gdy przed pomiarem XRF anality są zatężane ze znacznie większych objętości próbek (100-1000 mL) niż w opracowanych procedurach mikroekstrakcyjnych (5-75 mL).
- Nanocząstki węglowe dzięki dużej powierzchni właściwej mogą być zastosowane w dyspersyjnej ekstrakcji do fazy stałej w niewielkiej ilości, tj. 4 µg grafenu lub 10 µg nanorurek węglowych na 1 mL analizowanego roztworu. Po procesie zatężania możliwe jest więc uzyskanie cienkich próbek i w konsekwencji zaniedbanie efektów matrycy. Ponadto małe rozmiary nanocząstek pozwalają zminimalizować błędy związane z efektami ziarnistości w pomiarze EDXRF i TXRF.
- Procedury oparte na ekstrakcji do fazy stałej (DMSPE/EDXRF) charakteryzują się znacznie lepszą precyzją, RSD=2,6-5,1%, w porównaniu do procedur wykorzystujących

mikroekstrakcję ciecz-ciecz (DLLME/EDXRF), RSD=6,4-14,7%. Na całkowitą niepewność procedury DLLME/EDXRF największy wpływ mają błędy związane z etapem ekstrakcji i z rozdzielaniem faz ciekłych. Większe wartości RSD dla procedury DMSPE/TXRF (6.0-10.5%) związane są z oddzielaniem fazy stałej od analizowanego roztworu opartym na dekantacji (w procedurach DMSPE/EDXRF stosowano sączenie pod zmniejszonym ciśnieniem).

- Procedury oparte na ekstrakcji do fazy stałej (DMSPE/EDXRF i DMSPE/TXRF) umożliwiają analizowanie próbek o wysokim zasoleniu, stwarzających szczególne trudności analityczne.
- Zastosowanie odczynnika chelatującego w procedurze (DMSPE/EDXRF) pozwala dodatkowo na przeprowadzenie analizy specjacyjnej selenu, a zwiększenie objętości analizowanej próbki do 75 mL obniżenie granicy wykrywalności do 0,03 ng mL^{-1}.
- Pomiar technikami EDXRF i TXRF oraz minimalne zużycie odczynników chemicznych sprawiają, że opracowane procedury analityczne są w pełni zgodne z zasadami zielonej chemii analitycznej.

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9. PRZEDRUKI PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

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PAPER

Liquid-phase microextraction as an attractive tool for multielement trace analysis in combination with X-ray fluorescence spectrometry: an example of simultaneous determination of Fe, Co, Zn, Ga, Se and Pb in water samples

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In recent years, liquid-phase microextraction (LPME) has become one of the most valuable techniques for the preconcentration and separation of trace and ultratrace elements. LPME can be combined with an atomic technique which requires only a few microlitres of liquid to perform a measurement, *e.g.* electrothermal atomic absorption spectrometry. In this study, combining LPME with X-ray fluorescence spectrometry (XRF), a dried-spot technique is proposed. Since the X-ray beam can be focused on a small spot size and simultaneously LPME produces a very small drop of a volume ranging from 2 to 30 μL, the combination of these two techniques is a very promising tool for multielement trace and ultratrace analyses. The present research was performed using dispersive liquid–liquid microextraction (DLLME) with APDC as a chelating agent. Nevertheless, any LPME technique, *e.g.* single-drop microextraction (SDME), can be applied in combination with XRF. Because XRF measurement follows the microextraction, deposition and drying of the small drop, the influence of the diameter of the dried residue on the intensity of fluorescent radiation is discussed in detail. Pipetting and spray-on techniques are proposed for the deposition of the small drop onto the substrate (membrane filter or Mylar foil). Under the optimized conditions, the detection limits were 2.8, 1.6, 2.5, 1.7, 2.1 and 4.1 ng mL^{-1} for Fe, Co, Zn, Ga, Se and Pb, respectively, with a preconcentration factor of 250 for 5 mL of the water sample.

Introduction

In recent years, increased interest in the development of environmentally friendly analytical procedures according to the rules of green chemistry has been observed. Replacing toxic reagents and the miniaturization of classical sample preparation techniques are unquestionable trends in analytical chemistry. Because of these trends, liquid-phase microextraction (LPME) has become one of the most valuable alternative techniques to classical liquid–liquid extraction. Microextraction was initially used for analyzing gaseous samples, but today it is being successfully applied for analyzing a wide variety of samples.[1–3] Although, most LPME techniques concern organic analysis, the number of papers devoted to inorganic trace and ultratrace analysis is increasing rapidly. The development of different modes of LPME and the application of these techniques in inorganic trace analysis have recently been reviewed.[4–6] Among modern LPME techniques applied to determine metal concentrations, the following can be listed: single-drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME), dispersive liquid–liquid microextraction based on solidification of a floating organic drop (DLLME-SFO) and hollow fiber liquid–liquid microextraction (HF-LLME).

Determining trace and ultratrace elements requires combining LPME with an adequate atomic spectrometry technique. Microextraction was employed in elemental analysis for the first time by Chamsaz *et al.*[7] Headspace-SDME was combined with electrothermal atomic absorption spectrometry (ETAAS) for the extraction and determination of arsenic in aqueous samples. Generally, LPME can be combined with an atomic technique which requires only a few microlitres of liquid to perform a measurement. For this reason ETAAS[8–12] and electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS)[13–15] are usually used with LPME. Recently, total reflection X-ray spectrometry (TXRF) has also been proposed for determining trace selenium in soils using DLLME.[16] On the other hand, spectroscopic techniques commonly applied in most laboratories such as flame atomic absorption spectrometry (FAAS), ICP-OES or ICP-MS are less suitable in combination with LPME. Although electrothermal atomization systems have advantages over the above-mentioned techniques, FAAS is also applied with LPME due to its simplicity as well as its low operation costs.[17–20] The combination of LPME and atomic spectrometry techniques has undergone significant growth in the last

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few years, especially since 2008.[21] ETAAS is definitely the most commonly applied technique in combination with LPME. This is primarily due to three factors: compact benchtop instruments are available in many laboratories due mainly to their relatively low cost, very low detection limits can be obtained and, more importantly, ETAAS requires only a few microlitres of liquid.

ETAAS is not the only suitable technique for the analysis of a small volume sample. Another spectroscopic technique which seems to be perfect for combining with LPME is X-ray fluorescence spectrometry (XRF), especially micro-beam XRF (μ-XRF). This technique has been popularized very rapidly due mainly to the development of X-ray optics and the availability of benchtop μ-XRF instruments on the market over the past few years. To date μ-XRF has been commonly applied for the nondestructive investigation of cultural heritage materials, electronic components, individual particles, biological samples, *etc.*[22] The detection limits of medium- and heavy-Z elements in these materials are usually at a 1–100 μg g^{-1} level. Thus, detection limits expressed in relative units cannot be considered to be very low. This results from the fact that the volume of a sample from which the X-ray fluorescence radiation originates is very small. For the same reason the classical preconcentration methods are not suitable for μ-XRF. After co-precipitation or liquid–liquid extraction trace elements are collected onto the filter of a relatively large area and conventional XRF spectrometry is more suitable for studying these elements.[23–25] A much more appropriate method of sample preparation for μ-XRF is the dried-spot technique which consists of depositing small amounts of liquid samples onto a thin-film substrate and drying it into a solid residue. Elements can even be detected at the pg level using this technique, although detection limits strongly depend on the X-ray source and X-ray optics that are applied.[26,27] However, the small size of the dried spot combined with spatially resolved excitation offers very low detection limits in trace elemental analysis of aqueous solutions.[28–30]

In this study, for the first time to the best of our knowledge, the application of LPME for multielement determination of trace elements using semi-μ-XRF spectrometry (900 μm focal spot size) is proposed. Since a X-ray beam is focused onto a small spot size and simultaneously LPME produces a very small drop of a volume ranging from 2 to 30 μL, the combination of these two techniques is a very promising tool for multielement trace and ultratrace analyses. The goal of the present research is to show that combining LPME with μ-XRF spectrometry allows very low detection limits to be obtained in a very simple, non-time consuming and low-cost way.

Experimental section

Instrumentation

The measurements were performed using a laboratory-constructed energy-dispersive X-ray fluorescence spectrometer (EDXRF). The air-cooled side-window Rh target X-ray tube of *ca.* 100 μm nominal focal spot size (XTF 5011/75, Oxford Instruments, USA) supplied by an XLG high-voltage generator (Spellman, USA) was used as the excitation source. The X-ray tube was operated at 50 kV and 1400 μA. A monocapillary of *ca.* 900 μm focal spot size was used for measurements of the dried residue. The X-ray spectra emitted by the sample were collected using a thermoelectrically cooled Si-PIN detector (XR-100CR Amptek, Bedford, MA, USA) with a 145 eV resolution at 5.9 keV that was coupled to a multichannel analyzer (PX4 Amptek, Bedford, MA, USA). The spectrometer is described in detail in ref. 31 The deconvolution of X-ray spectra was performed using XRF-FP Amptek software.

Reagents and materials

Stock solutions (1 mg mL^{-1} of Fe, Co, Zn, Ga, Pb and Se), nitric acid (65%, Suprapur®), and ammonium hydroxide solution (25%, Suprapur®) were purchased from Merck (Darmstadt, Germany), and ammonium pyrrolidine dithiocarbamate—APDC (>98%), carbon tetrachloride (p.a.), and methanol (p.a.) were purchased from POCh (Gliwice, Poland). The pH of the solutions being analyzed was adjusted with 0.1 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NH_3. A standard multi-element solution (50 μg mL^{-1} of Fe, Co, Zn, Ga, Pb and Se) was prepared from the stock solutions (1 mg mL^{-1}). All reagents were dissolved and diluted with high purity water obtained from a Milli-Q system.

Sample preparation

Dispersive liquid–liquid microextraction (DLLME). Trace elements were preconcentrated using the DLLME procedure with APDC as chelating agents, carbon tetrachloride as the extraction phase and methanol as the disperser agent. The analytical procedure is presented in Fig. 1. In order to perform DLLME, 5 mL of the solution being analyzed was placed in a glass test tube. The pH of the sample was adjusted to 3 using 0.1 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NH_3. Then 100 μL of 5% (m/v) APDC solution was added. In the next step, a mixture of 30 μL of carbon tetrachloride and 500 μL of methanol was rapidly injected into the aqueous sample. At this stage, a cloudy solution containing many dispersed fine droplets of carbon tetrachloride was formed and the determined metal ions were extracted as the APDC complexes. The mixture was centrifuged for 10 min at 4000 rpm. After this process, the dispersed fine droplets were sedimented at the bottom of the test tube. The volume of the sedimented phase was *ca.* 20 μL. 10 μL of the sediment was then collected using a microsyringe and deposited onto the thin substrate.

The blank sample was prepared using the described procedure and high purity water obtained from the Milli-Q system instead of the analyzed solution.

Preparation of calibration samples. The calibration samples were prepared using the described DLLME procedure using a series of multielement solutions containing elements in amounts ranging from 0.01 to 2 μg.

Deposition of the organic phase onto the carrier. The organic phase can be deposited on a carrier by pipetting or by using the spray-on technique. In the first case, the organic phase can be pipetted onto the miniature filters of diameters, *e.g.*, 1.5 mm. The residue of a very small diameter can also be obtained if the organic phase is placed on the filter of classical diameter (*e.g.* 25 or 47 mm) in very small volumes, *e.g.* 1 μL, and immediately

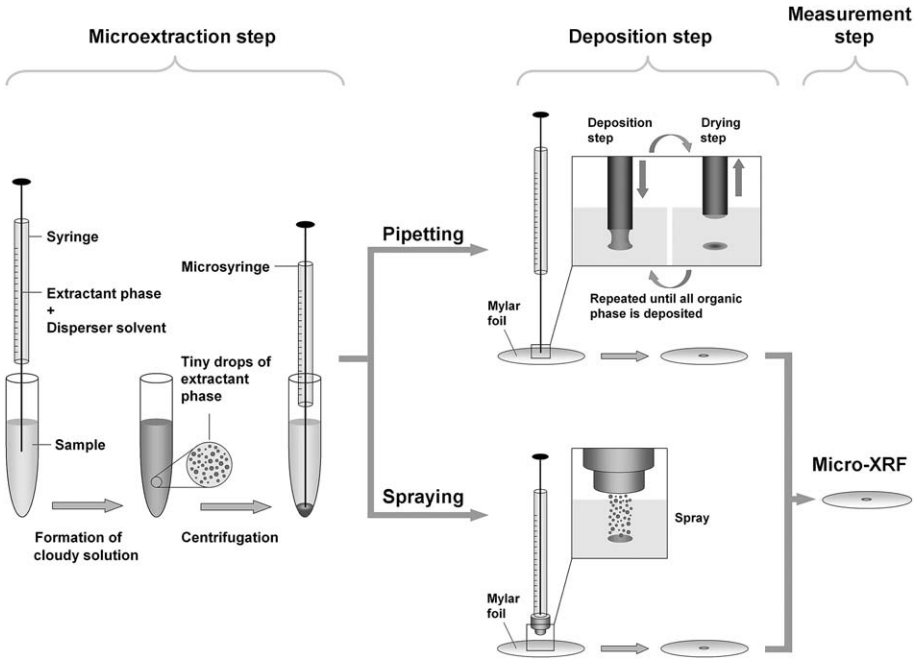

Fig. 1 Scheme of the DLLME procedure applied for XRF measurement.

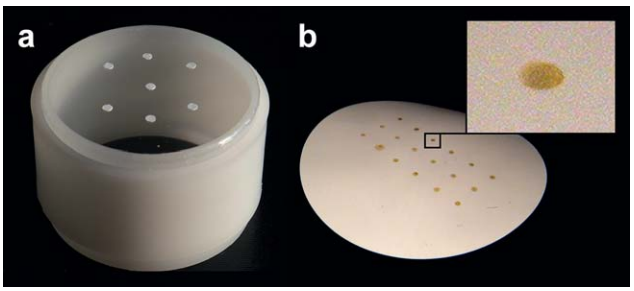

Fig. 2 Photograph of small filters placed on Mylar foil (a) and micro-samples prepared on a Millipore filter (b).

dried. In the second case, a stream of gas carries the sample from the cannula tip onto the carrier. For the spray-on technique, a Desaga HPTLC Applicator As 30 with a 10 μL dosing microsyringe was used. Both the Millipore filter and Mylar foil can be used as a sample carrier. It is worth emphasizing that the set of calibration samples and unknown samples can be prepared on one Millipore filter as presented in Fig. 2 and can then be measured many times.

Results and discussion

The present study demonstrates the first application of LPME for the multielement determination of trace elements using μ-XRF spectrometry. The μ-XRF measurement follows the microextraction and deposition of a small drop from the extraction phase onto the carrier and then drying it. The diameter of the dried residue on the carrier has a great influence on the analysis. Thus this issue, which is directly connected with the specificity of the μ-XRF measurement, will be discussed first. In the next part of this section the influence of some parameters on DLLME in view of multielement analysis will be presented.

Influence of the diameter of the dried spot on the intensity of radiation

In order to obtain a satisfactory signal-to-background ratio, only a thin carrier such as a membrane filter or Mylar foil can be used. Some sample support films for μ-XRF analysis were evaluated by Wilson *et al.*[32] Nevertheless, a low background is not the only issue in the analysis of the dried residue using μ-XRF. The second issue is the diameter of the dried residue. In general, the diameter of the dried residue should be small enough when the measurements are performed using the X-ray beam of a small spot size. If the area of the dried residue is too large, only a small fraction of the extracted elements is excited and in consequence

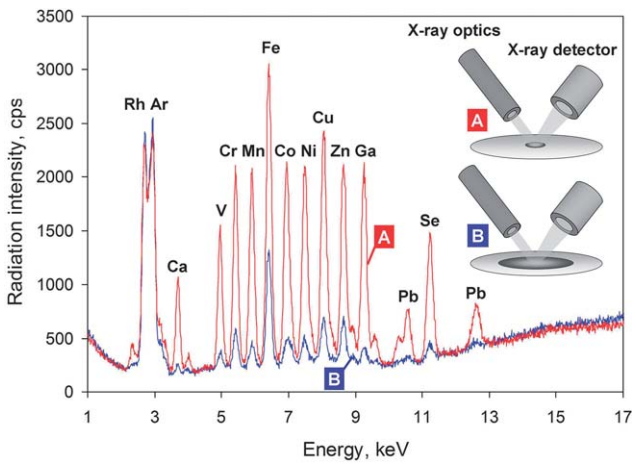

Fig. 3 EDXRF spectra of two reference samples containing 0.5 μg of each element. Samples A and B of various diameters (*ca.* 1 and 5 mm, respectively) deposited onto the Millipore filter are excited with an X-ray beam of 900 μm focus spot size.

a low intensity of fluorescent radiation is observed. Fig. 3 presents the EDXRF spectra of two samples deposited onto Millipore filters and excited using an X-ray beam of a 900 μm focal spot size. The samples of various diameters, *i.e.* 1 and 5 mm, contain the same amount of each element (0.5 μg). As can be observed, when the diameter of the sample is small, a high intensity of fluorescent radiation is detected. In view of the importance of this issue in μ-XRF analysis, the influence of the diameter of the dried residue on the intensity of fluorescent radiation will be discussed in some detail. For the LPME procedure, two cases can be considered here.

- In the first one, the chelating agent is added to the sample solution before extraction (*e.g.* APDC). Then, the complexes that are formed are extracted into droplets of the organic phase.

- In the second case, the chelating agent is added to the organic phase (*e.g.* dithizone) and then the extraction is performed.

The main difference between these two strategies regarding μ-XRF measurement is the concentration of the chelating agent in the organic phase and the total mass of the dried residue. The theoretical relationships between the intensities of the fluorescent radiation of various elements and the diameter of the dried residue are presented in Fig. 4. X-Ray beam spot size (Gaussian function is assumed) and absorption of X-ray radiation in the dried residue were considered in the calculations. Each sample has the same mass, *i.e.* if the diameter is smaller the thickness of the residue is higher. At the same time, absorption effects play a more important role. Fig. 4a presents the first case, when APDC is added to the sample solution and the complexes that are formed are extracted into droplets of the organic phase. Thus, the concentration of APDC in the extraction phase is low and depends on the concentration of metal ions in the sample. The intensities of radiation decrease with the diameter of the dried residue, which results from the fact that the fraction of excited elements decreases, or in other words, the masses per unit area of the determined element decrease while the X-ray beam spot size remains unchanged. For a small diameter of the residue the absorption effects are considerable and lower intensities of elements emitting low-energy radiation can be observed. Moreover, a very similar intensity of radiation (in relative units) for various elements can be observed if the diameter of the residue is high enough, which results from low absorption of characteristic radiation. Much more significant absorption effects can be observed if the chelating agent is present in the organic phase (*e.g.* 250 μg of dithizone in 30 μL of carbon tetrachloride, *i.e.* 0.8% (m/v) solution). This situation is illustrated in Fig. 4b. The significant difference between the cases illustrated in Fig. 4a and b (APDC and dithizone) can be observed for small diameters of residues. As can be observed in Fig. 4b, if the diameter of the residue is small enough, the fluorescent radiation decreases with a decrease in its diameter (or an increase in the thickness of the residue). This can be explained by the fact that the residue reaches a critical thickness and fluorescent radiation does not increase with thickness, and simultaneously, the area of the residue emitting radiation decreases.

In general, the diameter of the dried residue should be as small as possible, particularly if the chelating agent is added directly to the sample and the complexes that are formed are extracted into the organic phase. On the other hand, if the chelating agent is present in the organic phase in a relatively high concentration, some absorption effects can be observed, especially for elements emitting low-energy radiation. In practice, residues of diameters from 500 to 1500 μm can be obtained relatively easily for liquids of a volume ranging from 5 to 30 μL. Such residue diameters are suitable for measurements using a spectrometer with a monocapillary of *ca.* 900 μm focal spot size. Commercially available benchtop μ-XRF instruments frequently allow the focal spot size to be adjusted (*e.g.* from 30 μm to 2 mm), thus measurement conditions can be optimized. Dried residues can also be analyzed using instruments that give a very small focal spot size, *e.g.* 30 μm. The loss of the intensity of fluorescent radiation resulting from a small fraction of excited elements is compensated for by using polycapillary optics that reach gains of up to 10 000.

Deposition of the organic phase onto the carrier

The problem of keeping a liquid sample within a small spot also occurs in total-reflection X-ray fluorescence analysis (TXRF), where an aqueous solution of between 5 and 50 μL is pipetted onto a carrier. When a nonhydrophobic carrier, such as quartz, is used, the surface should be coated with a silicone solution to keep the aqueous sample within a small spot of a few millimetres in diameter.[33,34] In dried spot μ-XRF analysis several techniques such as microwave-assisted drying[35] or drying in flowing hot nitrogen[36] have been proposed to reduce the time of sample

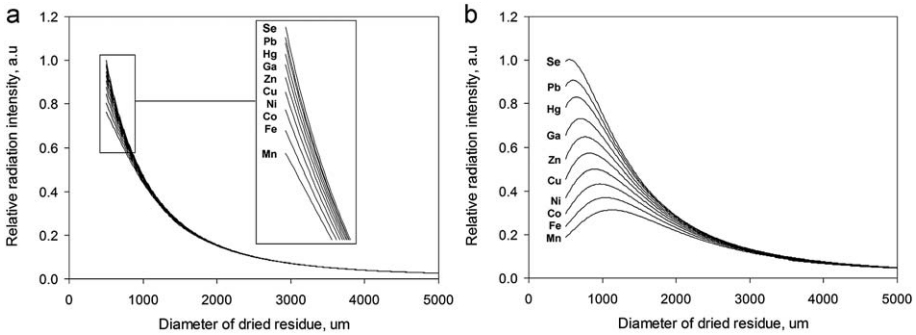

Fig. 4 Influence of the diameter of the dried residue on fluorescent radiation intensities of various elements extracted using: (a) APDC and (b) dithizone. The samples are excited using an X-ray beam of 900 μm focal spot size. Dried residues of various diameters contain the same amounts of the chelating agent (6.1 μg of APDC or 250 μg of dithizone) and each element (0.1 μg). Masses per unit area of the sample of diameter from 500 μm to 5000 μm vary from 3.8 to 0.04 μg cm^{-2} for APDC and from 130 to 1.3 μg cm^{-2} for dithizone. Lα line for Hg and Pb, Kα for others.

drying and to improve the precision of sample preparation. In both TXRF and dried spot μ-XRF analyses, the aqueous solutions are deposited. In these cases, the droplet stays within a small spot because of the high surface tension of water. In our case, residues of diameters ranging from 1000 to 1500 μm cannot be obtained by pipetting 5–30 μL of the organic phase onto a Millipore filter or Mylar foil because the deposited droplets may spill. In contrast to TXRF, a hydrophilic carrier cannot be applied because of the low surface tension of carbon tetrachloride. Thus, even if the organic phase is pipetted onto a hydrophilic carrier (or coated with a hydrophilic agent), the droplet of the organic phase does not stay within a small spot. To solve this problem, we propose the deposition of the organic phase onto a carrier by pipetting or by using the spray-on technique. In the first case, the organic phase should be placed on the filter in very small volumes, *e.g.* 1 μL, and immediately dried. Then residue of a very small diameter can be obtained. In the second case, a stream of gas carries the sample from the cannula tip onto the carrier. The spray-on technique is used in thin-layer chromatography and it is precise and suitable for routine use.

Optimization of analytical procedure, recovery and detection limits

Until now LPME has been used for the preconcentration and determination of single elements (usually in combination with ETAAS). In this study LPME was used for multielement trace analysis in combination with μ-XRF spectrometry. Thus, the analytical procedure should be optimized by taking into account multielement analysis rather than the determination of a single element. First of all a nonspecific reagent should be selected as the chelating agent. The present research demonstrates the application of DLLME for the simultaneous determination of Fe, Co, Ga, Zn, Se and Pb. In order to obtain a high extraction efficiency of these elements, APDC was used as the chelating agent, although other nonspecific reagents such as sodium diethyldithiocarbamate (NADDTC), 1-(2-pyridylazo)-2-naphthol (PAN), 8-hydroxychinoline or dithizone can be used. The DLLME procedure was performed with carbon tetrachloride as the extraction phase and methanol as the disperser agent. These solvents in amounts of 30 μL and 500 μL, respectively, were selected because they form a stable cloudy solution.[37,38] In the first step, the influence of the pH value of the sample (5 mL containing 0.5 μg of each element) on recovery as a critical issue in LPME was investigated. The effect of pH on the extraction in a pH range from 1 to 11 is presented in Fig. 5. For Fe and Pb the best recovery can be obtained in a pH range from 3 to 5, for Co and Zn from 3 to 6, for Se from 2 to 5 and for Ga from 2 to 3. For multielement analysis, a pH of 3 was selected for the subsequent work and for the analysis of a real sample. It should be noted that if Ga is not to be determined, a pH from 3 to 5 can be used. For pH above 5 the recovery of Fe, Se and Pb rapidly decreases. The recoveries obtained for pH = 3 were 97 ± 8, 95 ± 6, 92 ± 12, 99 ± 13, 95 ± 6 and 93 ± 7% for Fe, Co, Zn, Ga, Se and Pb, respectively. Using the recommended procedure allows a high enrichment factor of 250 to be obtained. The volume of the phase sedimented from a sample of 5 mL volume was 20 μL. 10 μL of this sedimented phase were then removed using a microsyringe and deposited onto a Millipore filter. Under these conditions

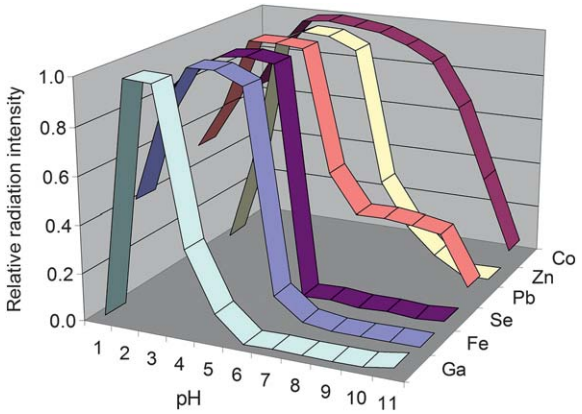

Fig. 5 Influence of pH on extraction of selected elements by DLLME using APDC as the chelating agent, carbon tetrachloride as the extraction phase and methanol as the disperser agent.

detection limits of 2.8, 1.6, 2.5, 1.7, 2.1 and 4.1 ng mL^{-1} were obtained for Fe, Co, Zn, Ga, Se and Pb, respectively (the detection limit was calculated from $DL = (3/k)(R/t)^{1/2}$, where k is the sensitivity of the method, R is the background count rate in counts s^{-1} and t is the counting time).

Precision

Because DLLME was combined with the μ-XRF measurement, the repeatabilities of various stages of the proposed procedure were evaluated. The total uncertainty of the analysis consists of many errors whose sources are the extraction step, the deposition of the organic phase onto the carrier and μ-XRF measurement. The uncertainty of μ-XRF measurement results from the errors associated with the positioning of the sample, instrument instability and counting statistics. The relative standard deviation (RSD) characterizing instrument instability and counting statistics equaled 1–1.3% for Fe, Co, Zn, Ga, and Se and 2.6% for Pb in this study. The higher value of RSD for Pb results from the lower intensity of $L_{\alpha 1}$ line as compared to the $K_{\alpha 1}$ lines that were applied for the determination of Fe, Co, Zn, Ga and Se. Because some inhomogeneity of the deposited sample can be expected, the precision of the positioning of the X-ray beam was evaluated by repositioning the same sample. RSD characterizing the precision of the positioning of the sample equaled *ca.* 5.1%. The precision of the deposition of the sample onto the carrier by the spray-on technique was *ca.* 3.6%. Both the precision of the positioning and deposition can be considerably improved by using an internal standard. If cobalt that was extracted together with other metals was used as the internal standard, the RSD characterizing the precision of the positioning and deposition of the sample was *ca.* 2.5%. A serious source of uncertainty can be the extraction step. In our case, the RSDs of the extraction of Fe, Co, Zn, Ga, Se and Pb were 7.1, 3.1, 13.1, 13.0, 3.0 and 4.4%, respectively. The high values of RSDs for Zn and Ga can be explained by the extraction conditions, *i.e.* pH adjusted to 3. Below this value the recovery of Zn and Ga decreases rapidly. Thus, a small deviation in pH can reduce the precision of the extraction step. Finally, the total RSDs for the determination of trace elements using a combination of DLLME and μ-XRF

spectrometry were 9.3, 7.5, 14.7, 14.0, 7.5 and 9.0% for Fe, Co, Zn, Ga, Se and Pb, respectively.

Calibration and analysis of unknown samples

The calibration samples were prepared using the described DLLME procedure using a series of multielement solutions. In our preliminary study, the calibration was performed in the range from 0.01 to 5 μg of each element. Nevertheless, above 2 μg of each analyte non-linear relationships between fluorescent radiation intensity and analyte concentration were observed. The exemplary results for Co are presented in Fig. 6. The non-linear relationship can be explained by the self-absorption effects increasing with the mass (thickness) of the dried residue. Nevertheless, the precipitation of APDC complexes plays a central role in this phenomenon. In other words, if the determined elements are present in a comparatively high concentration then precipitation is observed, determined metal ions are not extracted into the organic phase as APDC complexes, and in consequence recovery decreases. Thus, the proposed methodology can be applied for a metal concentration up to 0.4 μg mL^{-1} of Fe, Co, Ga, Se, Zn and Pb (2 μg in 5 mL of the analyzed solution).

The reliability of the combination of DLLME and μ-XRF spectrometry was examined through an analysis of river water. The accuracy of the analytical procedure was verified by an analysis using the water sample spiked with a known amount of the elements to be determined. The sample was spiked with 0.02 μg mL^{-1} of Co, Ga, Se, and Pb and 0.2 μg L^{-1} of Fe and Zn. The results presented in Table 1 show that the recoveries of the determined elements are in the range of 95–110%. Reliability was also checked using the AAS technique. Fe and Zn were determined using FAAS without sample preparation, whereas Co and Pb were separated and preconcentrated using solid phase extraction and then determined by ETAAS. As can be seen, the relative differences between AAS and μ-XRF are in the range of 6–11%. The results demonstrate that the proposed combination of DLLME and μ-XRF is suitable for the analysis of real water samples.

Conclusions and future perspectives

The combination of LPME and μ-XRF is a very promising tool for multielement trace and ultratrace analyses. This is the result of two factors: an X-ray beam of high intensity is focused onto a small spot size and LPME produces a very small drop of a volume ranging from 2 to 30 μL, which can be dried to a small spot. Using this combination multiplies the capabilities of both LPME and μ-XRF. First of all, a very high enrichment factor can be reached using LPME. Secondly, using the μ-XRF dried-spot technique significantly improves the signal-to-background ratio due to the reduction in the background by focusing the X-ray beam onto the size of the dried residue, by applying a thin sample carrier, and finally by removing the light matrix. The theory and experiment show that the best signal-to-background ratio can be achieved if the X-ray beam spot is matched to the diameter of the dried residue. Then a high fraction of extracted elements is excited and simultaneously the background resulting from the scattering from the sample carrier is low. In practice, a dried sample of a very small diameter can be obtained by pipetting and step-by-step drying or by using the spray-on technique. The present research shows that combining LPME with μ-XRF spectrometry allows low detection limits to be obtained in a very simple, non-time consuming and low-cost

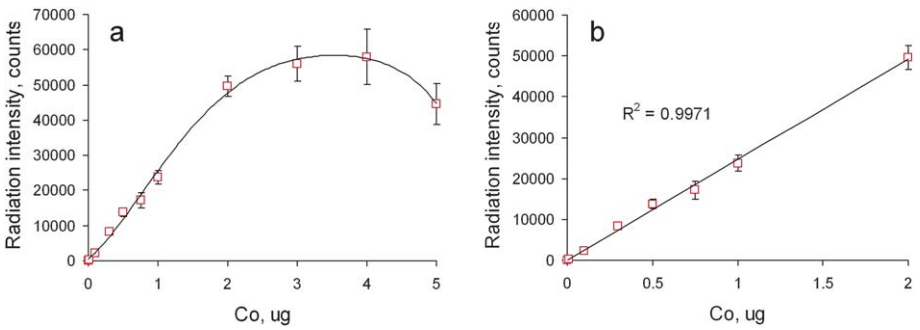

Fig. 6 Relationship between fluorescent radiation intensity and the mass of extracted Co ranging from 0.01 to 5 μg (a) and from 0.01 to 2 μg (b).

Table 1 Analysis of river water and samples spiked with 0.02 μg mL^{-1} of Co, Ga, Se, and Pb, and 0.2 μg mL^{-1} of Fe and Zn

	DLLME with μ-XRF			AAS	
Element	River water/μg mL^{-1}	Spiked river water/μg mL^{-1}	Recovery (%)	River water/μg mL^{-1}	Relative differences (%)
Fe	0.25 ± 0.02	0.44 ± 0.03	95	0.236 ± 0.004	5.9
Co	0.042 ± 0.003	0.062 ± 0.003	100	0.039 ± 0.001	7.8
Zn	0.16 ± 0.02	0.38 ± 0.04	110	0.18 ± 0.01	11.1
Ga	<DL	0.019 ± 0.003	95	n.d.	—
Se	<DL	0.022 ± 0.001	110	n.d.	—
Pb	0.041 ± 0.003	0.062 ± 0.005	105	0.046 ± 0.006	10.9

way. The advantage of this combination over ET-AAS is the possibility of performing multielement analysis. Moreover, unknown samples and more importantly calibration samples can be nondestructively measured with high precision many times. However, in comparison with ET-AAS, μ-XRF has lower sensitivity and higher detection limits.

The present research was performed using dispersive liquid–liquid microextraction (DLLME) with APDC as the chelating agent. However, any LPME technique, such as SDME or DLLME-SFO, can be applied in combination with μ-XRF. The recently proposed advanced nanolitre or even picolitre dried-spot sample preparation systems designed for μ-XRF measurement in combination with LPME are promising tools, particularly for very small organic drops.[39–41] Because μ-XRF measurement is easy to automate, the implementation of LPME on a lab-on-chip system with μ-XRF is very promising for microanalysis. Papers dedicated to microextraction on lab-on-chip systems for organic analysis have been recently published.[42–44] Due to the trend toward the miniaturization of sample preparation techniques, developing such systems for inorganic trace and ultratrace analysis can be expected. Previously, Tsuji *et al.* presented a chemical microchip suitable for measurements using μ-XRF.[45] The sample solution is dried and concentrated in a suitable area corresponding to the size of the primary X-ray beam. In the future, such lab-on-chips with microchannels for mixing, chemical reactions and separation may be very promising tools for a precise and fast determination of trace elements on a nanolitre scale using LPME and μ-XRF as the final detection technique.

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Analytical Note

Dispersive liquid–liquid microextraction using diethyldithiocarbamate as a chelating agent and the dried-spot technique for the determination of Fe, Co, Ni, Cu, Zn, Se and Pb by energy-dispersive X-ray fluorescence spectrometry

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Water sample

ABSTRACT

Dispersive liquid–liquid microextraction (DLLME) using sodium diethyldithiocarbamate (DDTC) as a chelating agent was investigated for the simultaneous determination of iron, cobalt, nickel, copper, zinc, selenium and lead ions in water samples. The procedure was performed using 5 mL of the sample, 100 μL of a 0.5% solution of DDTC, 30 μL of carbon tetrachloride (extraction phase) and 500 μL of methanol (disperser solvent). The experiments showed that Fe, Co, Ni, Cu, Zn and Pb can be simultaneously extracted at a pH of 5 and that Se can be extracted at a pH of 2–3. The results were compared with those obtained using ammonium pyrrolidine dithiocarbamate as a chelating agent. For all analytes, a linear range was observed up to 0.4 μg mL^{-1}. If Fe and Zn are present in concentrations 10 times higher than those of the other analytes, then the linearity is observed up to 0.2 μg mL^{-1}. In the present study, the organic phase that contained preconcentrated elements was deposited onto a Millipore filter and measured using energy-dispersive X-ray fluorescence spectrometry. The obtained detection limits were 2.9, 1.5, 2.0, 2.3, 2.5, 2.0 and 3.9 ng mL^{-1} for Fe, Co, Ni, Cu, Zn, Se and Pb, respectively. This combination of DLLME and the dried-spot technique is promising for multielement analyses using other spectroscopy techniques, such as laser ablation-inductively coupled plasma-mass spectrometry, laser-induced breakdown spectroscopy or total-reflection X-ray fluorescence spectrometry.

1. Introduction

X-ray fluorescence spectrometry (XRF) is a fast, simple and relatively inexpensive detection technique that enables multielemental analysis of different types of materials. However, direct XRF analysis of liquids does not allow the determination of trace and ultratrace elements. Therefore, an appropriate preconcentration procedure is necessary before the XRF analysis, e.g., evaporation, co-precipitation, solid-phase extraction, electrodeposition or classical liquid-phase extraction (LPE) [1]. Techniques based on the miniaturization of LPE, i.e., liquid-phase microextraction (LPME) [2–6], can also be a very promising tool for trace and ultratrace XRF analysis. LPME is usually combined with a spectroscopy technique that requires only a few microliters of organic phase to perform a measurement, e.g., electrothermal atomic absorption spectrometry (ETAAS) [7–10], electrothermal vaporization-inductively coupled plasma-mass spectrometry [11,12] and electrothermal vaporization-inductively coupled plasma-optical emission spectrometry [13]. These techniques do not require dilution of the extractant phase for the measurement, so the obtained enrichment factors are high and range from 50 to 400. Other techniques, such as flame atomic absorption spectrometry (FAAS) [14,15] and inductively coupled plasma-optical emission spectrometry [16,17], require more than a few microliters of extractant phase for the measurement; as a consequence, the extractant phase must be diluted, and the obtained enrichment factors are significantly decreased to approximately 15–150.

In the aforementioned spectroscopy techniques, the extractant phase of the micro-volume is directly injected into a small graphite tube, or the extractant phase is diluted and subsequently aspirated by a nebulizer and transformed into an aerosol. However, another strategy is to deposit a small volume of the extractant phase onto a substrate, dry the extractant into a solid residue and perform measurements using a suitable spectroscopy technique, e.g., energy-dispersive X-ray fluorescence spectrometry (EDXRF) [18], total-reflection X-ray fluorescence spectrometry (TXRF) [19] or laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) [20].

In this study, sodium diethyldithiocarbamate (DDTC) was used as a chelating agent for the multielemental determination of heavy metals using dispersive liquid–liquid microextraction (DLLME) as a preconcentration technique. Trace elements that were preconcentrated, deposited and dried in the form of small spots were detected using EDXRF. The combination of DLLME, the dried-spot technique and a suitable detection technique enables the possibility of multielemental analysis with very low detection limits. The possibility

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of performing multielemental analysis greatly reduces the amount of toxic, flammable and volatile organic solvents and wastes. These factors make the method less harmful to the environment. Moreover, the costs and time of the analysis and its laboriousness are significantly reduced.

2. Experimental

2.1. Chemicals and solutions

Stock standard solutions (1 mg mL^{-1} of Fe, Co, Ni, Cu, Zn, Ga, Pb and Se), nitric acid (65%, Suprapur®), ammonium hydroxide solution (25%, Suprapur®) were purchased from Merck (Darmstadt, Germany). DDTC (p.a.), carbon tetrachloride (p.a.) and methanol (p.a.) were purchased from POCh (Gliwice, Poland). All aqueous solutions were prepared using high-purity water obtained from Milli-Q water purification system. A solution of 0.5% DDTC was prepared by dissolving an appropriate amount of this reagent in pure methanol. The pH levels were adjusted with 0.1 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NH_3 solutions.

2.2. Apparatus

A laboratory-constructed EDXRF spectrometer described in detail in Ref. [21] was used for measurements. An air-cooled side-window Rh-target X-ray tube (XTF 5011/75, Oxford Instruments, USA) with a monocapillary with a ca. 900 μm focal spot size was used for measurements of the dried residues. The X-ray spectra emitted by the sample were collected using a thermoelectrically cooled Si-PIN detector (XR-100CR) with a resolution of 145 eV at 5.9 keV that was coupled to a multichannel analyzer (PX4, Amptek). The X-ray tube was operated at 45 kV and 1400 μA. The counting time was 600 s.

2.3. Dispersive liquid-phase microextraction procedure

The metal ions were preconcentrated from 5 mL of the sample using 100 μL of a 0.5% solution of DDTC (chelating agent), 30 μL of carbon tetrachloride (extraction phase) and 500 μL of methanol (disperser solvent). The DLLME procedure is described in detail in Ref. [18]. After centrifugation, 20 μL of organic phase was sedimented. Afterwards, 10 μL of the sedimented phase was deposited onto a Millipore filter using the spray-on technique based on the use of a Desaga HPTLC Applicator As 30. In the spray-on technique, a stream of gas carries the sample from the cannula tip onto the carrier. Deposition of the sample was performed with the use of a syringe with a capacity of 10 μL. The sample was deposited at the rate of approximately 60 μL·s^{-1} over 10 cycles with intervals of approximately 15 s. The Millipore filter was subsequently placed between two sheets of Mylar foil of 6 mm thickness mounted to the sample cup, and then EDXRF spectrometry was performed.

3. Results and discussion

In this study, DDTC was applied as a chelating agent for the simultaneous determination of Fe, Co, Ni, Cu, Zn, Se and Pb ions using DLLME as a preconcentration technique. After the extraction process, the organic phase that contained the determined elements was deposited onto a membrane filter in the shape of a spot. The application of the dried-spot technique in conjunction with EDXRF measurements allows the simultaneous determination of numerous trace elements. This strategy has an advantage over the combination of ETAAS and LPME, in which only a single element can be determined. Because of the multielement capability of the analysis, nonspecific chelating agent, such as DDTC [22,23], was selected. The application of a proper type and amount of extraction phase as well as a disperser solvent is crucial in DLLME. In this work, 30 μL of carbon tetrachloride (as an extraction solvent) and 500 μL of methanol (as a disperser solvent) were selected because they form a stable cloudy solution [24,25].

The most important factor with respect to the metal-chelating agent formation and extraction efficiency is the acidity of the sample solution. The effects of the solution pH on the formation of metal–DDTC complexes and on the extraction of analytes from water solutions were investigated within the pH range of 1 to 11; the pH levels of the sample solutions were adjusted with 0.1 mol L^{-1} HNO_3 or 0.1 mol L^{-1} NH_3. The results obtained in our previous work [18] for ammonium pyrrolidine dithiocarbamate (APDC) and those from present work obtained for DDTC are presented in Fig. 1. The previously obtained results show that, in the case of APDC, the extraction process for Fe, Co, Zn, Ga, Se and Pb ions is achieved with high recoveries. In contrast to APDC, DDTC used as a chelating agent allows the determination of Cu and Ni with very good recoveries and a high enrichment factor. However, Ga cannot be determined if DDTC is used as the chelating agent. In the case of DDTC, the best recovery is observed for Fe in the pH range from 4 to 5, for Co from pH 4 to 6, for Ni from pH 5 to 6, for Cu from pH 4 to 5, for Se from pH 2 to 3, for Pb from pH 4 to 7, and for Zn from pH 5 to 7. Given the previously discussed results and in view of the multielement nature of the analysis, a pH of 5 was selected for further studies in the case of DDTC. The pH of 5 selected for DDTC is adequate for six of the seven analytes: Fe, Co, Ni, Cu, Zn and Pb. These elements can be determined with recoveries of 93 ± 9, 99 ± 6, 95 ± 12, 94 ± 10, 93 ± 7, and 101 ± 8%, respectively. Se was not included in the subsequent analytical procedure because the extraction efficiency decreases rapidly at pH levels greater than 4. If a pH of 4 is selected for the determination of Se, then the quantification of Fe, Co, Cu and Pb is possible with very good recoveries. Notably, if DDTC is used as the chelating agent at a pH of 2, Se can be separated from other elements present in the environmental samples in large amounts, i.e., Fe and Zn. In summary, high recoveries of heavy-metal ions were obtained with a high enrichment factor of approximately 250 (5 mL of sample and 20 μL of enriched organic phase).

The total uncertainty in the analysis using DLLME combined with the dried-spot technique consists of many errors associated with the extraction step ($s_{extraction}$), the deposition of the organic phase onto the carrier ($s_{deposition}$), the positioning of the sample in the spectrometer chamber ($s_{positioning}$), and the measurement ($s_{measurement}$):

$$s_{total}^2 = s_{extraction}^2 + s_{deposition}^2 + s_{positioning}^2 + s_{measurement}^2.$$

The relative standard deviations (RSD_{total}), which characterize the total precision of DLLME using DDTC combined with the dried-spot technique and EDXRF spectrometry, were approximately 9.8, 6.4, 12.8, 10.4, 7.1 and 7.8% for Fe, Co, Ni, Cu, Zn and Pb, respectively. Our previous work [18] showed that the extraction process made the greatest contribution to the uncertainty in the DLLME–EDXRF technique. The $RSD_{extraction}$ values that characterize the extraction step using DDTC were 7.3, 2.3, 11.5, 8.9, 4.0 and 4.2% for Fe, Co, Ni, Cu, Zn and Pb, respectively. In the case of Zn, the precision of the extraction is three times better for DDTC than for APDC [18]. For other metal ions, the precisions are similar.

The calibration curves were constructed using multielement standard solutions for the concentrations of analytes in the range from 0.002 to 1 μg mL^{-1}. The linear relationship between the radiation intensity and the analyte concentration was observed up to 0.4 μg mL^{-1}. Radiation intensity does not increase or even decreases at analyte concentrations greater than 0.4 μg mL^{-1}. Such relationships can be explained by the precipitation of DDTC complexes when the concentrations of analytes are relatively high (greater than 0.4 μg mL^{-1}). The precipitated complexes are then not extracted into the organic phase, and the recovery simultaneously decreases. Because Fe and Zn are usually present in environmental samples at concentrations higher than those of other elements, a new series of multielement standard solutions was prepared. In this case, Co, Ni, Cu, Se and Pb were present in the concentration range from 0.002 to

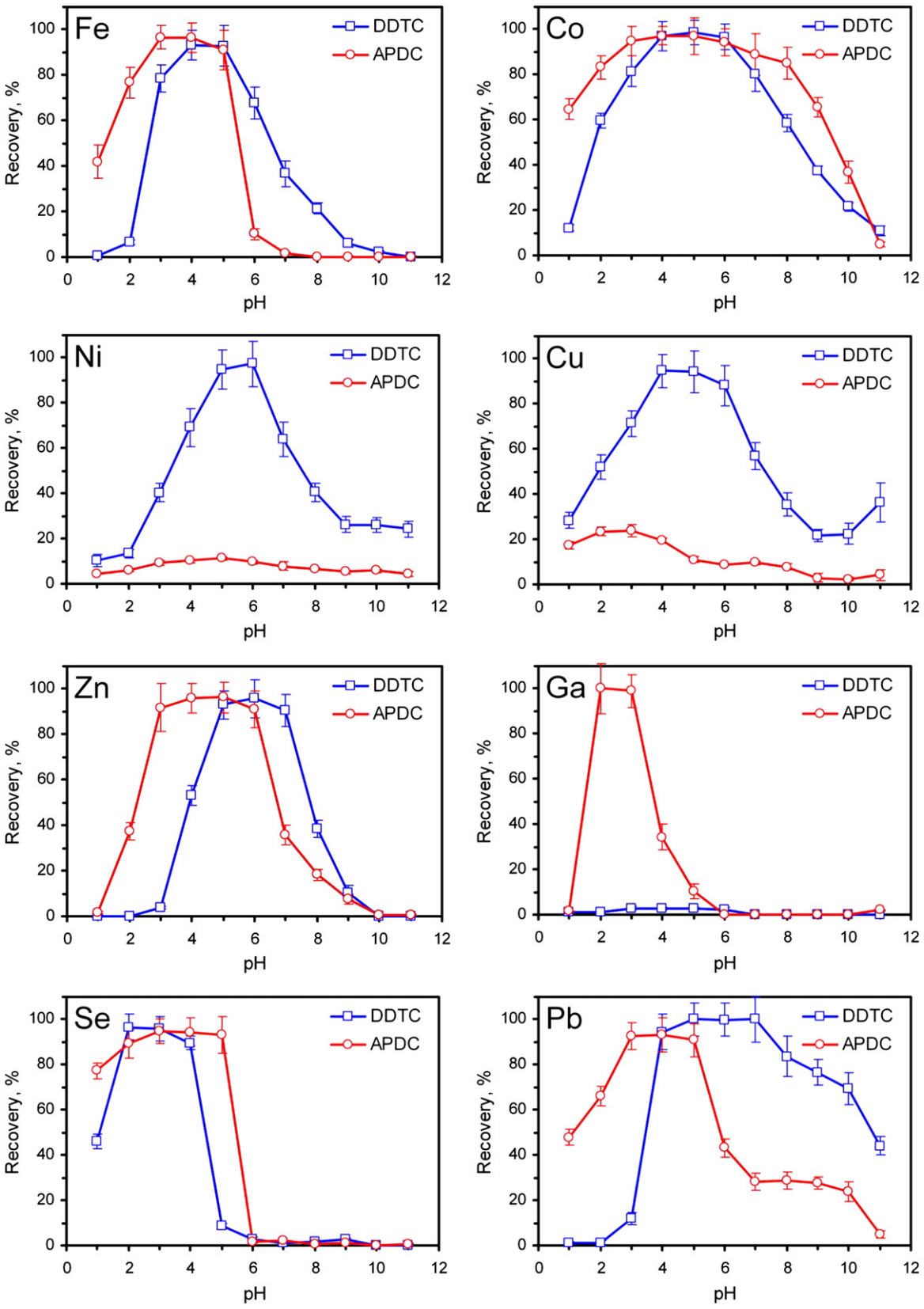

Fig. 1. The influence of pH on the recovery of elements preconcentrated by DLLME using DDTC and APDC as chelating agents (uncertainties correspond to one standard deviation, n = 3).

1 μg mL^{-1}, whereas Fe and Zn were present in the range from 0.02 to 10 μg mL^{-1}. In this case, the linear relationship between the intensity of the radiation and the analyte concentration was observed up to 0.2 μg mL^{-1}. The narrower range of linearity can be explained by the co-precipitation of analytes with Zn-DDTC and Fe-DDTC complexes. Detailed information on the calibration parameters (e.g.,

Table 1
Comparison of the developed DLLME–EDXRF strategy with recently reported analytical procedures for the determination of metal ions in water samples by EDXRF.

Elements	Preconcentration method	Sample volume, mL	Detection limits	Sample	Ref.
Fe, Cu, Zn, Se, Pb	Precipitation with APDC	300	From 2.0 ng mL^{-1} Zn to 6.0 ng mL^{-1} Fe	Water	[27]
Fe, Co, Ni, Cu, Zn, Se, Pb	Precipitation with APDC	300	From 1.0 ng mL^{-1} Zn to 2.4 ng mL^{-1} Pb	River water	[28]
Co, Cu, Zn	Solid-phase extraction using PAR[a] immobilized on anion-exchange resin	100	From 0.2 ng mL^{-1} Zn to 1.5 ng mL^{-1} Co	Seawater	[29]
Pb	Solid-phase extraction using Dowex 50 cation-exchange resin	100	1.5 ng mL^{-1} Pb	Tap water	[30]
Fe, Co, Ni, Cu, Zn, Pb,	Precipitation with APDC	1000	From 0.6 ng mL^{-1} Ni to 1.7 ng mL^{-1} Fe	Rain water	[31]
Fe, Co, Ni, Cu, Zn, Ga, Se, Pb	DLLME	5	From 1.5 ng mL^{-1} Co to 4.0 ng mL^{-1} Pb	River water	This work

[a] 4-(2-Pyridylazo)resorcinol.

slope, intercept, and correlation coefficient) is presented in the Supplementary materials.

The detection limits (DLs) for the developed analytical procedure were calculated from the equation $DL = (3/k)(R/t)^{1/2}$, where k is the sensitivity of the applied method, R is the background count rate (s^{-1}), and t is the counting time [26]. The obtained detection limits were 2.9, 1.5, 2.0, 2.3, 2.5, 2.0 and 3.9 ng mL^{-1} for Fe, Co, Ni, Cu, Zn, Se and Pb, respectively. Table 2 shows a comparison of the proposed DLLME–EDXRF methodology with other preconcentration methods combined with EDXRF measurements for the determination of metal ions in water samples. Although the DLs of the listed methods are, in all cases, in the ng mL^{-1} range, the proposed DLLME–EDXRF combination requires a significantly lower sample volume to perform the measurement. As is evident from the results in Table 1 and from the results summarized in a review paper [1], obtaining a DL in the ng mL^{-1} range requires a high volume of sample, from 100 to 1000 mL. Moreover, the other preconcentration methods listed are rather laborious and require the use of large amounts of organic solvents, which are flammable, volatile and toxic. The small volume of the sample applied in this work reduces the time required for the analysis and the volume of the consumed reagents, both of which significantly reduce the costs of the analysis. Thus, the proposed DLLME–EDXRF combination belongs to environmentally benign procedures according to green chemistry rules [32]. Moreover, the lower sample volume can be especially useful when the amount of the material to be analyzed is limited.

The proposed multielement DLLME combined with the dried-spot technique and EDXRF measurements was applied in the analysis of river water samples. The proposed procedure was verified using atomic absorption spectrometry (AAS). Fe and Zn were determined using flame atomic absorption spectrometry (FAAS, whereas the other elements were determined using the ETAAS technique. The results presented in Table 2 show that the proposed combination of DLLME and the dried-spot technique is suitable for the simultaneous determination of trace elements in real water samples.

4. Conclusions

LPME produces a very small volume of an organic phase that contains preconcentrated trace elements. Therefore, LPME must be combined with a spectroscopy technique that requires only a few microliters of liquid to perform a measurement. For the same reason, LPME is usually applied to determine a single element. The results presented here show that nonspecific chelating agents, such as DDTC, can be employed in multielemental analysis using the DLLME procedure. In the present work, although the trace elements preconcentrated, deposited and dried in the form of small spots were detected using EDXRF spectrometry, other spectroscopy techniques that allow the measurement of solid samples, e.g., TXRF, LA-ICP-MS or laser-induced breakdown spectroscopy, may also be successfully applied. The proposed combination of DLLME and the dried-spot technique may represent a basis for a new analytical strategy for the simultaneous determination of trace and ultratrace elements using techniques that allow measurements of solid samples.

Table 2
Determination of various elements in a water sample using EDXRF and AAS (uncertainties correspond to one standard deviation, n = 3).

	DLLME–EDXRF, μg mL^{-1}	AAS[a], μg mL^{-1}	Relative difference (%)
Fe	0.24 ± 0.03	0.235 ± 0.004	2.1
Co	0.045 ± 0.003	0.039 ± 0.001	15.4
Ni	0.035 ± 0.005	0.040 ± 0.001	−12.5
Cu	0.038 ± 0.004	0.035 ± 0.001	8.6
Zn	0.18 ± 0.01	0.19 ± 0.01	−5.3
Pb	0.048 ± 0.003	0.049 ± 0.006	−2.0

[a] Fe and Zn were determined using FAAS, whereas Co, Ni, Cu and Pb were determined by ETAAS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sab.2012.05.003.

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Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples

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In this paper the combination of dispersive micro solid-phase extraction (DMSPE), using multiwalled carbon nanotubes (MWCNTs) as solid sorbents, with total-reflection X-ray fluorescence spectrometry (TXRF) is proposed for preconcentration and determination of lead and cadmium ions in water samples. The proposed sample preparation is quite simple and economic. After the sorption processes of the metals on the MWCNTs, the aqueous sample is separated by centrifugation and the metal loaded MWCNTs are suspended using a small volume of an internal standard solution and analyzed directly by TXRF. Parameters affecting the extraction process (complexing agent, pH of the aqueous sample, amount of MWCNTs) and TXRF analysis (volume of the deposited suspension on the reflector, drying mode, and instrumental parameters) have been carefully evaluated to test the real capability of the developed methodology for the determination of Cd and Pb at trace levels. For both elements the linear range is observed up to 50 ng mL^{-1}. Under optimized conditions detection limits are 1.0 ng mL^{-1} and 2.1 ng mL^{-1} for Cd(II) and Pb(II) ions, respectively. Both of the examined elements can be determined with quantitative recoveries (*ca.* 100%) and with an adequate precision (RSD = 6.0% and 10.5% for Cd(II) and Pb(II), respectively). Our results give insight into the possibilities of the combination of DMSPE and TXRF for trace metal determination in different types of environmental waters (sea, river and waste water).

Introduction

The direct determination of metal ions by instrumental techniques is often limited due to the low concentration levels of the analytes and the presence of matrix interferences. Therefore, sample preparation is usually necessary to separate the analytes from complex matrices or to preconcentrate them in order to improve sensitivities and detection limits. Unfortunately, this step is considered the most time-consuming and error-prone step of the whole analytical procedure. Moreover, the classical sample pretreatment techniques such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) require high volumes of toxic reagents. In recent years, increased interest in the development of environmentally friendly analytical procedures according to the rules of green chemistry has been observed.[1] The objectives of green analytical methods are replacing toxic reagents, minimizing waste in laboratory and in consequence miniaturization of classical methods. Because of these trends, liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) have become the most valuable alternative techniques to classical LLE and SPE.[2]

SPE has been extensively used for the preconcentration of metals in environmental waters.[3–5] In general, SPE is a surface dependent process since its kinetics depends directly on the contact surface between the analytes and the solid sorbent. This issue becomes critical when the amount of solid sorbent is reduced to the microscale. In this context, dispersive-based procedures have gained importance as rapid and efficient sample treatment methodologies.[6] In dispersive solid phase extraction (DSPE) and in dispersive micro solid-phase extraction (DMSPE) the solid sorbent in the μg or mg range is dispersed in the analyzed solution. Such a strategy promotes the immediate interaction between the analytes and sorbent and shortens the time of sample preparation. After adsorption the analytes held in the solid sorbent are eluted,[7–9] thermally desorbed[10] or directly determined by a suitable spectroscopic technique.[11–14]

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The nature and properties of the solid sorbent are of prime importance in DMSPE. In practice, the main requirements for a solid sorbent are: (a) the fast and quantitative sorption and elution, (b) a high surface area and high capacity, and (c) high dispersibility in liquid samples. In this context, nanoparticles (NPs) seem to be perfect for use in DMSPE. In general, NPs can be divided into two general groups according to their chemical nature: carbon-based, such as fullerene, carbon nanotubes (CNTs) and graphene, and inorganic NPs including magnetic NPs.[15,16] Such NPs can be applied in organic[7,8,17] and inorganic[18] analyses.

In previous studies we used multiwalled carbon nanotubes (MWCNTs) as solid sorbents in DMSPE procedures for the determination of heavy metal ions[12–14] by X-ray fluorescence spectrometry (XRF). Using this technique, the direct quantification of metal species held in solid materials is possible and therefore after the sorption process the loaded MWCNTs with metals were collected onto a filter and analyzed directly by XRF without the need for an additional elution step. In this paper, the combination of DMSPE using CNTs with total-reflection X-ray fluorescence spectrometry (TXRF) for the determination of Cd(II) and Pb(II) is proposed. TXRF is a well established analytical technique for multi-element determination in various sample types, especially liquids and powdered or micro-samples.[19] To perform analysis under total-reflection conditions, samples must be provided as thin films. For liquid samples, this is done by depositing 5–50 μL of the sample on a reflective carrier and subsequently drying the drop. The TXRF system makes use of the fact that at very low glancing angles of the primary X-ray beam (~0.1°) the primary beam is totally reflected. Therefore, the high background that would generally occur due to scattering from the sample support is absent leading to improved detection limits compared to conventional XRF systems.[20] Taking into account the micro-analytical capability of TXRF and the possibility to analyze suspensions, the use of MWCNTs as solid sorbents is presented for the first time as an interesting preconcentration strategy to be combined with TXRF spectrometry. Moreover, the very small particle size of MWCNTs made these solid sorbents appropriate to prepare a representative suspension to be analyzed by TXRF, thus avoiding the extraction step. Parameters affecting the extraction process by DMSPE (complexing agent, pH of the aqueous sample, amount of MWCNTs) and TXRF analysis (volume of the deposited suspension on the reflector, drying mode, and instrumental parameters) have been carefully evaluated to test the real capability of the developed DMSPE + TXRF methodology for the determination of low levels of Cd(II) and Pb(II) ions in water samples.

Experimental

Reagents, materials and solutions

Stock solutions of 1000 mg L^{-1} of Cd(II), Pb(II) and Y used for preparation of standard solutions and spiked samples were purchased from Teknolab (Drobak, Norway). All aqueous solutions were prepared using high-purity water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA). The complexing agent, ammonium pyrrolidine dithiocarbamate (APDC, >99%) was purchased from Sigma-Aldrich (Madrid, Spain). Concentrated nitric acid (65%, Suprapur) and ammonium hydroxide (25%, Suprapur) used to prepare solutions of which pH was adjusted were purchased from Merck (Darmstadt, Germany). MWCNTs with diameters of 6–9 nm and lengths of *ca.* 5 mm were purchased from Sigma-Aldrich (Steinheim, Germany). The procedure used in this paper for the oxidation of the MWCNTs is described in detail in ref. 13. In this work quartz glass discs with a diameter of 30 mm and a thickness of 3 mm ± 0.1 mm were used as sample holders for introducing the sample into the TXRF instrument.

To test the real capability of the combination of DMSPE and TXRF spectrometry for the determination of Pb(II) and Cd(II), the developed procedure was applied to the analysis of different types of environmental waters including sea, river and waste water samples. All of the analyzed samples were filtered through Millipore filters (0.45 μm) and preserved by the addition of an appropriate volume of nitric acid and stored at 4 °C.

Table 1 Instrumental parameters and measurement conditions

S2 PICOFOX TXRF benchtop spectrometer
Tungsten target X-ray tube
Rating: 50 kV, 1 mA (maximum power 50 W)
Optics: multilayer Ni/C, 17.5 keV, 80% reflectivity
Detector: Si drift detector, 10 mm^2, <160 eV resolution Mn-K_α
Working environment: air
Sample station: cassette changer for 25 samples
Measurement time: 2000 s

Instrumentation and operating conditions

TXRF analysis was performed using a benchtop spectrometer S2 PICOFOX™ (Bruker AXS Microanalysis GmbH, Berlin, Germany). The spectrometer specifications and operating conditions used are summarized in Table 1. This instrument is equipped with a tungsten target X-ray tube that allows performing TXRF analysis using K-lines of high atomic number elements such as Cd. An additional advantage of this spectrometer compared to other existing systems is that it uses an air-cooled low-power X-ray tube and a Peltier cooled silicon drift detector and thus, no cooling media and gas consumption are required.

The evaluation of TXRF spectra and calculation of net peak areas of the analytes were performed using the software (Spectra Plus 5.3, Bruker AXS Microanalysis GmbH, Berlin, Germany) linked to the equipment.[21] For the quantification in TXRF analysis, the software applies a deconvolution routine which uses measured mono-element profiles for the evaluation of peak areas.

Preconcentration procedure and TXRF analysis

The developed DMSPE procedure was as follows: an aliquot of 20 mL of sample was placed in a 25 mL polypropylene plastic conical centrifuge tube. Then, 100 μL of 4 mg mL^{-1} APDC and 100 μL of 2 mg mL^{-1} oxidized-MWCNT suspension were added.

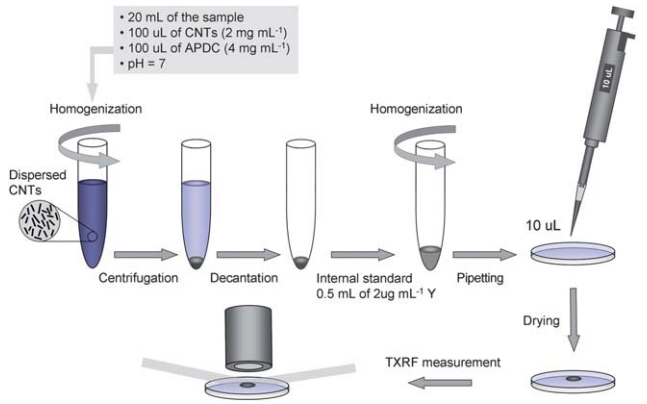

Fig. 1 Schematic setup for the DMSPE procedure combined with TXRF analysis.

The pH of the sample was adjusted to 7 using 0.1 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NH_3. Subsequently, the solution was shaken for 5 min. Homogenization was immediately achieved, which promoted the interaction between the Cd(II)–APDC, Pb(II)–APDC complexes and oxidized-MWCNTs. The mixture was centrifuged for 20 min at 4000 rpm. After this process, the dispersed MWCNTs with adsorbed complexes were sedimented at the bottom of the test tube. The clear liquid was then poured off carefully. Then, 500 μL of 2 μg mL^{-1} Y in 2 mol L^{-1} HNO_3 (internal standard) was added to the sedimented phase. After homogenization using a Vortex device an aliquot of 10 μL was transferred onto a siliconized quartz glass sample carrier and dried under an IR heater for subsequent TXRF analysis (Fig. 1).

Results and discussion

Evaluation of Pb and Cd extraction by DMSPE

In order to obtain high cadmium and lead preconcentration rates, the effect of different parameters affecting cadmium and lead extraction such as aqueous sample pH, aqueous sample volume, amount of APDC, amount of MWCNTs and the effect of extraction time were carefully evaluated. One variable at a time optimization was used to obtain the most favorable conditions for the DMSPE procedure.

Effect of sample pH

The acidity of the sample solution has a very important influence on the metal ion complexation process and therefore on extraction efficiency. The effect of the sample pH on recovery of extracted elements in the presence and in the absence of APDC was studied in the pH range of 1–12. As can be seen in Fig. 2, the application of APDC allows the extraction of Cd(II) and Pb(II) ions with high recoveries at a pH ranging from 2 to 12 and from 4 to 9, respectively. When APDC is not used for the complexation of metal ions the best recoveries are observed in a narrower pH range – from 5 to 12 in the case of Cd(II) and from 6 to 10 in the case of Pb(II). Taking into account the obtained results, a sample pH of 7 was selected for simultaneous extraction of Cd(II) and Pb(II) ions from the analyzed samples. Because the application of APDC improves the recoveries, the subsequent experiments were performed using a complexing agent.

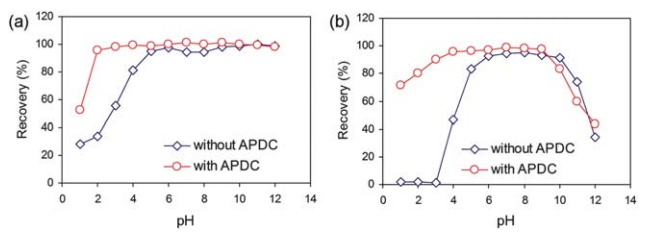

Fig. 2 The influence of pH on recovery of determined elements with and without application of APDC for Cd(II) (a) and Pb(II) (b) ions (sample volume 20 mL, 20 μg mL^{-1} of APDC, 10 μg mL^{-1} of MWCNTs, extraction time 5 min).

Effect of the sample volume

Another parameter that can significantly affect the global sensitivity of the methodology is the volume of the aqueous sample used for preconcentration. To evaluate this effect, two different sample volumes (20 mL and 40 mL) of an aqueous solution containing 0.4 μg of Cd and Pb were analyzed in duplicate. The results obtained showed a decrease in Cd(II) recoveries (*ca.* 86%) when using the higher sample volume. In addition, relative standard deviations (RSDs) calculated for Pb and Cd measurements were also higher when using 40 mL of the aqueous sample to perform the analysis (8% and 17% in comparison with 6% and 10.5% for Cd(II) and Pb(II), respectively). Therefore, a sample volume of 20 mL of the aqueous sample was selected for further experiments.

Effect of the amount of APDC

APDC allows the formation of hydrophobic complexes that can be absorbed on the MWCNT surface through van der Waals forces and hydrophobic interactions.[16] Therefore, the influence of the concentration of the complexing agent on the recovery of determined elements was investigated (see Fig. 3a). The results obtained showed that the APDC concentration has no impact on the efficiency of the developed analytical procedure within the studied range (5–30 μg mL^{-1}). Finally, an APDC concentration of 20 μg mL^{-1} was selected for subsequent experiments.

Effect of the amount of MWCNTs

The amount of MWCNTs used for preconcentration can have a significant influence on the extraction of metals from the aqueous solution but also on the later TXRF analysis. As stated in the Introduction section, to perform the analysis under total reflection conditions, analyzed samples must be deposited as thin layers on a reflective carrier. Therefore, the thickness of the deposited samples, which is related to the amount of MWCNTs used, can have influence on the final determination of our analytes. Fig. 3b depicts the influence of MWCNT amount (within the range of 2–20 μg mL^{-1}) on the recovery values for Pb(II) and Cd(II). As is shown, low Cd(II) and Pb(II) recoveries (*ca.* 50%) were obtained when using low MWCNT concentrations (2 μg mL^{-1}). Satisfactory recoveries (*ca.* 100%) for Cd(II) and Pb(II) ions were achieved for concentrations of MWCNTs ranging from 5 to 10 μg L^{-1}. With further increase of the absorbent amount, recovery values decreased considerably for both

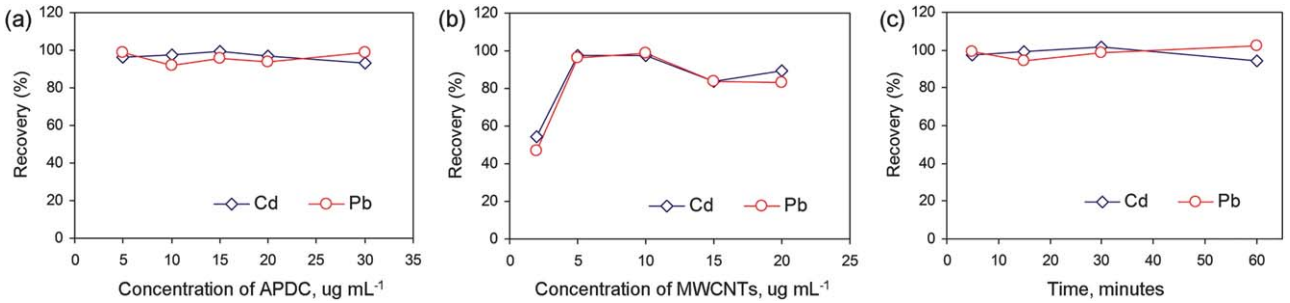

Fig. 3 The influence of some parameters on the determination of Cd(II) and Pb(II) ions by the DMSPE + TXRF procedure: (a) APDC concentration (pH = 7, sample volume 20 mL, 10 μg mL^{-1} of MWCNTs, extraction time 5 min), (b) MWCNT concentration (pH = 7, sample volume 20 mL, 20 μg mL^{-1} of APDC, extraction time 5 min) and (c) extraction time (pH = 7, sample volume 20 mL, 20 μg mL^{-1} of APDC, 10 μg mL^{-1} of MWCNTs).

determined elements. This fact demonstrated that probably when working with such a high amount of MWCNTs the thin layer is broken and thus the analysis cannot be performed under total-reflection conditions. In view of the obtained results, a MWCNT concentration of 10 μg mL^{-1} was established for further experiments.

Effect of extraction time

In DMSPE it is also of significance to establish an appropriate contact time (period between the addition of the sorbent to the analyzed sample and the centrifugation step) to obtain high analyte extraction rates. In Fig. 3c, the effect of extraction time on Cd(II) and Pb(II) recoveries is shown. As can be seen, the extraction time does not affect the determination of analytes in the studied working range (from 5 to 60 min). This fact can be related to the large contact area between dispersed MWCNTs and the aqueous sample that provides a fast achievement of the equilibrium state. The adsorption occurs immediately, and the extraction time has no influence on the adsorption of the metal–APDC complexes onto the surface of MWCNTs.[14] In order to reduce the analysis time of the developed methodology, a stirring time of 5 min was chosen for subsequent experiments.

Selection of analytical conditions for TXRF measurements

Parameters affecting the sample deposition step (sample deposition volume and drying mode) as well as operating conditions for TXRF measurements were evaluated to obtain the best sensitivity for Cd and Pb determination.

Influence of sample deposition volume

The aim of the sample preparation process in TXRF is to obtain the target sample as a thin layer (<100 μm) on a carrier with high reflectivity sample support. Therefore, the choice of adequate sample deposition volume is of crucial importance in order to obtain a thin layer and to ensure the conditions of total reflection. Moreover the diameter of the sample spot on the sample carrier has to be within the beam size to ensure the complete exposition of the drop to the X-ray beam. For that, only a few microliters of solution are usually employed. In Fig. 4a, the effect of sample deposition volume on Cd and Pb relative intensities (I_{Cd}/I_Y and I_{Pb}/I_Y) obtained for the analysis of a preconcentrated standard solution containing 20 ng mL^{-1} of both metals is shown. As can be seen, no statistically significant differences on relative analyte responses were obtained when depositing 5 or 10 μL of the sample on the reflector. A slight decrease of Cd and Pb relative intensities was observed when using higher sample deposition volumes. In view of the obtained results, a volume of 10 μL was established for further experiments.

Influence of sample drying

After the deposition procedure on the reflector, the microdroplet must be properly dried to perform the TXRF analysis. Therefore, another parameter that can significantly affect the final analytical TXRF results is the drying mode used. For that, different drying modes were tested in order to ensure the achievement of a centered-thin film on the reflector when

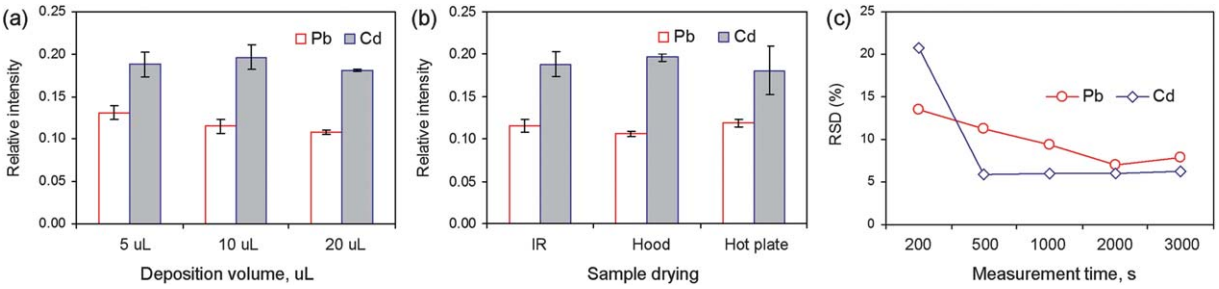

Fig. 4 The influence of: (a) sample deposition volume, (b) sample drying mode and (c) measurement time on the relative intensity of Cd(II) and Pb(II) ions by the DMSPE + TXRF procedure.

analyzing a preconcentrated standard solution containing 20 ng mL^{-1} of Cd and Pb: (i) drying under a laminar flow hood (room temperature), (ii) drying under an IR-lamp and (iii) drying on a hot plate set at ~80 °C. Results showed that for this type of deposited sample (suspension of loaded MWCNTs) the drying mode is not a critical parameter and no statistically significant differences were found for the different drying modes tested (see Fig. 4b). Finally, samples were dried under an IR-lamp for practical reasons.

Operating conditions for TXRF measurements

Operating conditions for TXRF measurements were also evaluated to obtain the best instrumental sensitivity for Cd and Pb determination. The rate of kV and mA of the X-ray tube was selected to work under conditions of maximum efficiency of excitation (50 kV, 1 mA, max. power 50 W). The measurement time was selected with respect to the lowest relative standard deviation (RSD) obtainable as detailed in Fig. 4c. Each experimental point represents the RSD value calculated from the analysis of five replicates of a deposited preconcentrated sample containing 20 ng mL^{-1} of Cd and Pb. As expected, the higher the integration time the lower the standard deviation until a constant value was reached (RSD ~5 to 10%). From these results, a measurement time of 2000 s was fixed.

Analytical figures of merit of the DMSPE + TXRF system

First of all, a study was conducted to study the benefits of the proposed preconcentration methodology for Cd and Pb determination by TXRF. In Fig. 5, the comparison between TXRF spectra obtained for the direct analysis of an aqueous standard solution containing 20 ng mL^{-1} of Cd and Pb and after the DMSPE + TXRF procedure is shown. It is clear that the proposed DMSPE preconcentration method offers obvious benefits in terms of sensitivity compared to analysis of aqueous samples without preconcentration. This fact is particularly interesting when using portable TXRF systems that offer extreme simplicity of operation in a low-cost compact design (no cooling media or gas consumption are required for operation) but they also present limited sensitivity compared with high-scale instrumentation. If the parameters for TXRF spectra shown in Fig. 5 (net intensity and background) are used to estimate the detection limits (DLs) according to the 3σ approach,[20] values of approximately 1.0 ng mL^{-1} for Cd(II) and 2.1 ng mL^{-1} for Pb(II) are obtained. These values are below the maximum contaminant levels for Cd(II) (5 ng mL^{-1} (ref. 22)) and Pb(II) (15 ng mL^{-1} (ref. 23)), that might be found in drinking water according to United States Environmental Protection Agency (EPA). Moreover, calculated DLs are below the Polish regulations for bottled water (3 ng mL^{-1} and 10 ng mL^{-1} for Cd(II) and Pb(II), respectively).[24]

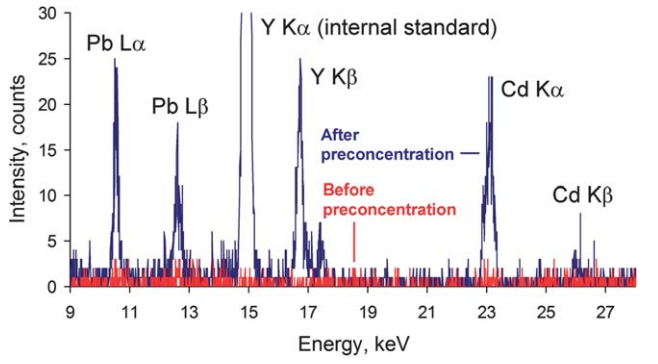

Fig. 5 Comparison between TXRF spectra obtained for the direct analysis of an aqueous standard solution containing 20 ng mL^{-1} (red line) and after the DMSPE + TXRF procedure (blue line).

Table 2 Calibration curve parameters and DLs for determination of Cd(II) and Pb(II) ions by combination of DMSPE and portable TXRF spectrometry. The concentration of Cd(II) and Pb(II) are in ng mL^{-1}

Analyte	Equation	R	DL, ng mL^{-1}
Cd	$I_{Cd}/I_Y = (0.00925 \pm 0.00023) \times C_{Cd} - (0.007 \pm 0.011)$	0.9988	1.0
Pb	$I_{Pb}/I_Y = (0.00434 \pm 0.0001) \times C_{Pb} + (0.013 \pm 0.006)$	0.9980	2.1

The linearity of the proposed analytical procedure was examined for the concentration of analytes in the range of 5–50.0 ng mL^{-1}. To reduce inaccuracies due to sample deposition on the sample carrier calibration using internal standardization was used. The obtained results show that the proposed methodology is linear for the entire range of examined concentrations. Calibration parameters (slope, intercept, and correlation coefficient) are listed in detail in Table 2.

The total uncertainty of the developed methodology (DMSPE + TXRF) consists of the errors associated with the extraction step, errors linked to the deposition of the sample onto the carrier and errors related to instrument and counting statistics from the TXRF system. In order to evaluate the extent of each one of these errors, five independent solutions containing 20 ng mL^{-1} of Cd and Pb were preconcentrated and measured under optimized conditions (total uncertainty). Besides, one of the preconcentrated samples was analyzed by the deposition on five different carriers (deposition of the sample on the carrier + instrumental errors) and one of the carriers was analyzed five times (instrumental errors). In all cases, the RSDs associated were calculated and values are presented in Table 3. As is shown, the combination of TXRF and DMSPE is characterized by satisfactory total uncertainty precision (RSD ~5 to 10%). It is also interesting to remark that the uncertainty associated with

Table 3 The relative standard deviations (RSDs) describing the precision of the extraction step, pipetting the sample onto the carrier, the measurement of analytes and the total uncertainty of the developed methodology

	RSD, %			
Analyte	Extraction	Pipetting	Measurement	Total uncertainty
Cd	4.2	3.4	2.6	6.0
Pb	9.6	2.6	3.2	10.5

Table 4 Analytical results (mean ± SD, $n = 3$) for cadmium and lead in water samples

Element	Sample	Added, ng mL^{-1}	Found, ng mL^{-1}	Recovery (%)
Cd	Sea water	0	5 ± 1.2	
		20	26 ± 3.6	105
		50	57 ± 3.6	104
	River water	0	3.9 ± 0.5	
		20	27 ± 3.0	116
		50	58.3 ± 0.9	109
	Waste water	0	3.4 ± 0.3	
		20	24 ± 4.5	103
		50	56 ± 2.3	105
Pb	Sea water	0	12 ± 1.8	
		20	32.2 ± 0.5	101
		50	67 ± 3.7	110
	River water	0	7 ± 1.6	
		20	30 ± 2.3	115
		50	58 ± 4.4	102
	Waste water	0	7 ± 1.5	
		20	28 ± 2.5	105
		50	53.5 ± 0.5	93

the extraction step is the greatest contribution to the total uncertainty of the DMSPE-TXRF system.

Analytical application

The proposed method for simultaneous determination of Cd(II) and Pb(II) by DMSPE-TXRF was applied to the analysis of different types of water samples including sea water, river water and waste water. The reliability of the developed procedure was examined by the analysis of water samples spiked with 20 and 50 ng mL^{-1} of Cd(II) and Pb(II). According to the results presented in Table 4, the recoveries for spiked water samples averaged 106% and 104% for Cd(II) and Pb(II), respectively. The highest RSDs were obtained for samples which did not contain the addition of the standard solution due to the proximity of the measured concentrations to the DLs. The results summarized in Table 4 show that the developed combination of DMSPE and TXRF is suitable for the simultaneous determination of Cd(II) and Pb(II) ions in real water samples, including sea water.

Conclusions

The results of the present investigation show that DMSPE combined with TXRF spectrometry is a powerful methodology for the determination of low levels of Cd(II) and Pb(II) ions in water samples. Application of MWCNTs in DMSPE provides a large contact area between the sorbent and analytes of interest, so the equilibrium state is achieved immediately and the extraction process is almost time independent. The use of TXRF as the detection system eliminates the need for an elution step after the adsorption process of the analytes on the MWCNTs, simplifying the procedure and reducing the total analysis time. Additional advantages of the TXRF method proposed are the possibility to detect simultaneously Cd and Pb ions in the sample and the low operating costs since the portable TXRF system used does not require cooling media and gas for operation. The achieved DLs are below the maximum Cd and Pb contents permissible in drinking waters according to EPA and Polish regulations for bottled water and the accuracy and precision of the obtained results are adequate for the intended purpose. The developed analytical methodology has been successfully applied for the determination of Cd and Pb in the low ng mL^{-1} range in different types of water samples.

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Trace and ultratrace determination of heavy metal ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid sorbent in dispersive micro solid-phase extraction

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ABSTRACT

In this paper, the adsorptive properties of graphene nanosheets were used for simultaneous preconcentration of cobalt, nickel, copper and lead ions from water samples. The developed methodology is based on dispersive micro-solid phase extraction (DMSPE) which is miniaturized and a simplified version of classical solid phase extraction technique. In proposed procedure only 200 μL of suspension containing graphene (0.2 mg), ammonium pyrrolidine dithiocarbamate (APDC) (0.8 mg) and Triton-X-100 (0.1 mg) is rapidly injected to 50 mL of water sample. Then, graphene nanosheets with adsorbed metal-APDC chelates are collected on membrane filter and measured using energy-dispersive X-ray fluorescence (EDXRF) spectrometry. The various parameters including pH, amount of APDC, sample volume, amount of Triton-X-100 and sorption time were optimized in order to obtain the best recoveries. The experiment shows that Co, Ni, Cu and Pb can be simultaneously preconcentrated at pH of 5 with high recoveries (97%, 96%, 99% and 96% for Co, Ni, Cu and Pb, respectively) and very good precision (RSDs within 2.6–3.4%). Due to the excellent enrichment factors ranging from 400 to 2500 the proposed DMSPE–EDXRF procedure offers low detection limits. For optimized measurement conditions (voltage and current of X-ray tube, primary beam filter) the detection limits are even 0.08, 0.07, 0.08 and 0.20 ng mL^{-1} for Co, Ni, Cu and Pb, respectively.

1. Introduction

During the last few years, the number of publications devoted to graphene significantly increased. Extensive studies revealed pattern of useful applications of that allotropic form of carbon including electronics [1], transistors [2], solar cells [3], energy storage [4–6], sensors [7], quantum electronics [8], composites [9] and biodevices [10,11]. One of the reasons of such intense research in that area is unique structure of that nanomaterial. Graphene consists of sp^2-hybrydized carbon atoms organized in regular hexagonal layers, which make it chemically stable, flexible, corrosion resistant and durable [12,13]. Graphene is characterized by a large surface area providing high adsorption capacity towards various molecules. It is noteworthy that in the case of graphene, both sides of planar sheets are responsible for molecule adsorption, whereas in the case of carbon nanotubes (CNTs) or fullerenes only exterior walls take part in this process due to the steric hindrance [14]. Adsorption properties are explained also by great delocalized π-electron system which plays a significant role in the formation of π–π stacking interaction with carbon-based ring structures [15]. This feature is interesting from the standpoint of analytical chemistry, where graphene has been recently applied for the preconcentration of organic compounds and trace elements [16,17]. Methodologies in which graphene is used most frequently are solid-phase extraction (SPE) and solid-phase microextraction (SPME). An overwhelming majority of publications is concerned on organic compounds, when graphene is applied as a solid sorbent. Only a few of papers is devoted to the issue of metal ions preconcentration. Graphene was successfully applied in classical SPE for preconcentration of Pb(II) in water and vegetable samples by flame atomic adsorption spectrometry (FAAS). In described methodology dithizone was used as a complexing agent [18]. Similar procedures were proposed for preconcentration of Cr(III) [19], Co(II) and Ni(II) [20] ions, where 8-hydroxyquinoline and APDC were used as complexing agents, respectively. The presence of chelating agent is essential, because metal ions in the form of hydrophilic complexes are not adsorbed on the graphene surface. Employment of chelating agent allows the formation of hydrophobic complexes that can be absorbed on the nanomaterial surface through the Van der Waals forces and hydrophobic interactions.

Although graphene has excellent adsorptive properties, practical application of that nanomaterial in classical SPE mode may encounter many difficulties. These problems result mainly from the very small size of graphene particles. Firstly, nanosheets may escape from the SPE cartridge. Secondly, graphene particles may redound to the

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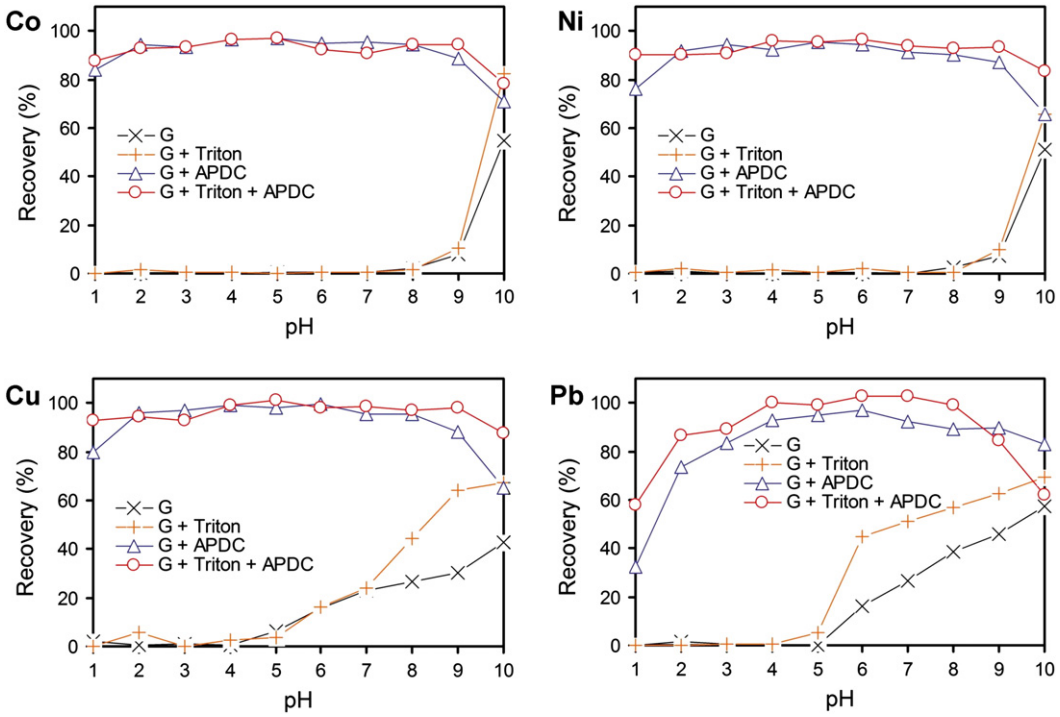

Fig. 1. The influence of pH on the determination of Co(II), Ni(II), Cu(II) and Pb(II) ions by the DMSPE–EDXRF procedure: (sample volume 50 mL, elements concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})); G is the abbreviation for graphene.

formation of high pressure inside the SPE system. However, the small size of graphene nanosheets which cause serious problems in classical SPE, can be considered as a great advantage in dispersive micro solid-phase extraction (DMSPE). In this paper DMSPE using graphene as a solid sorbent is applied for simultaneous preconcentration and determination of Co(II), Ni(II), Cu(II) and Pb(II) in water samples. The studies show that due to the high surface area and excellent adsorption capacity, graphene can be successfully used in a very small amount (i.e. 4 μg per 1 mL of analyzed solution) in DMSPE. In our previous work we have applied CNTs in DMSPE technique [21–24]. However, due to the steric hindrance the quantitative recovery of enriched elements could be obtained only if higher amount of solid sorbent (CNTs) was applied (10 μg per 1 mL of analyzed solution). In this approach, application of graphene in small amounts allows obtaining thin samples of small diameter. In consequence high signal to background ratio is observed and excellent detection limits (DLs) of enriched elements are obtained. To the best of our knowledge, graphene has been applied for the first time in combination with energy-dispersive X-ray fluorescence (EDXRF) spectrometry. Moreover, usage of chelating agent allows determining elements in a much more complex matrix in comparison to methodology utilizing graphene precursor, i.e. graphene oxide (GO) [25].

2. Experimental

2.1. Chemicals and solutions

Stock solutions (1 mg mL^{-1} of Co(II), Pb(II), Ni(II), Cu(II)), nitric acid (65%, Suprapur®), and ammonium hydroxide solution (25%, Suprapur®) were purchased from Merck (Darmstadt, Germany). A standard multi-elemental solution of 10 μg mL^{-1} was prepared from the stock solutions. The pH of the analyzed solutions was adjusted with 0.1 mol L^{-1} HNO_3 and/or 0.1 mol L^{-1} NH_3. Graphene of 8 nm thickness, ammonium pyrrolidine dithiocarbamate — APDC (>98%) and Triton-X-100 were purchased from Graphene Supermarket® (Calverton, USA), Acros Organics (Geel, Belgium) and POCh (Gliwice, Poland), respectively. All reagents were dissolved and diluted with a high purity water obtained from the Milli-Q system.

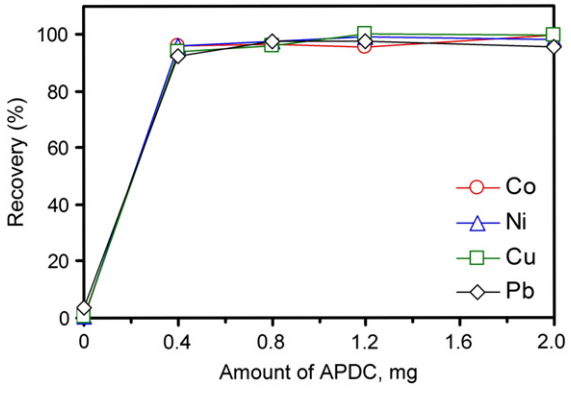

Fig. 2. Effect of APDC amount (pH = 5, sample volume 50 mL, element concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (0–10 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})).

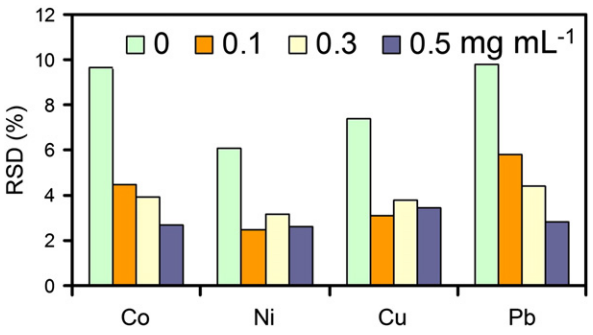

Fig. 3. Effect of Triton-X-100 amount (pH = 5, sample volume 50 mL, element concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0–0.5 mg mL^{-1})).

2.2. Preparation of graphene/APDC/Triton-X-100 suspension

Graphene/APDC/Triton-X-100 suspension was prepared as follows: mixture of 25 mg of graphene, 100 mg of APDC and 12.5 mg of Triton-X-100 was sonicated for 60 min in 25 mL of high purity water. In order to obtain homogenous graphene suspension it was additionally sonicated for 5 min before every application.

2.3. Sample preparation

All water samples were filtered through a Millipore cellulose acetate membrane (0.45 μm) and stored at 4 °C for no longer than 2 days. Artificial sea water was prepared as follows: 21.03 g NaCl, 3.52 g Na_2SO_4, 0.61 g KCl, 0.088 g KBr, 0.034 g $Na_2B_4O_7 \cdot 10H_2O$, 9.50 g $MgCl_2 \cdot 6H_2O$, 1.32 g $CaCl_2 \cdot 2H_2O$, 0.02 g $SrCl_2 \cdot 6\ H_2O$ and 0.02 g $NaHCO_3$ were dissolved in 1 L of high purity water [26].

2.4. Instrumentation

The measurements were performed using a laboratory-constructed EDXRF spectrometer. The air-cooled Rh target X-ray tube with 125 μm Be window and max. power of 75 W (XTF 5011/75, Oxford Instruments, USA) was used as an excitation source. The X-ray spectra emitted by the samples were collected using a thermoelectrically cooled Si-PIN detector with 12.5 μm Be window and 145 eV resolution at 5.9 keV (XR-100CR Amptek, Bedford, MA, USA). The spectrometer is described in detail in ref. [27]. The deconvolution of X-ray spectra was performed using XRF-FP Amptek software. The measurements were also performed using benchtop EDXRF spectrometer — Epsilon 3 (PANalytical, Almelo, The Netherlands) with a Rh target X-ray tube with 50 μm Be window and a max. power of 9 W. The spectrometer is equipped with thermoelectrically cooled silicon drift detector (SDD) with 8 μm Be window and a resolution of 135 eV at 5.9 keV. The system is also equipped with 10-position removable sample changer, spinner and five primary filters that can be selected to improve measuring conditions for determined elements.

2.5. Preconcentration procedure

Initially, the pH value of 50 mL sample solution was adjusted to 5 using 0.1 mol L^{-1} HNO_3 and/or 0.1 mol L^{-1} NH_3. Then, 200 μL of graphene/APDC/Triton-X-100 suspension (1 mg mL^{-1} graphene, 4 mg mL^{-1} APDC, 0.5 mg mL^{-1} Triton-X-100) was injected to the analyzed solution. In the next step, the whole volume of analyzed sample was passed through a membrane filter with the use of filtration assembly of 5 mm in diameter. Subsequently, graphene with adsorbed metal chelates collected onto the filter was dried under an IR heater and measured with the use of EDXRF spectrometry.

3. Results and discussion

In order to obtain the best recovery, precision and high enrichment factor the parameters such as pH, amount of APDC and Triton-X-100, and volume of the sample and time of the sorption process were optimized. One variable at time optimization was performed to choose the most convenient conditions for DMSPE–EDXRF procedure.

3.1. Effect of chelating agent and sample pH

The effect of sample pH on recovery of extracted elements was examined in the pH range of 1–10. In order to find the most suitable pH value for subsequent experiment, analytical procedure was performed using various reagents in different combinations:

- graphene,
- graphene and APDC,
- graphene and Triton-X-100,
- graphene, APDC and Triton-X-100.

Results depicted in Fig. 1 show that preconcentration based on graphene without chelating agent (APDC) is possible (but not quantitative) only above pH of 5 for Cu(II) and Pb(II) and over pH of 9 for Co(II) and Ni(II). However, this effect does not result from the sorption process itself, but is intimately intertwined with precipitation of hydroxides of determined elements. In fact, significant increase in recovery values is observed in the presence of chelating agent. With a view of further multielemental analysis, non-specific chelating agent, e.g. APDC, was chosen for subsequent experiment. APDC allows formation of hydrophobic complexes which in that form are adsorbed on the graphene surface. Results presented in Fig. 1 show that high recovery (close to 100%) can be obtained in broad range of pH, i.e. from 2 to 9 for Co(II), Ni(II) and Cu(II) and from 4 to 9 for Pb(II). Unfortunately, we observed the low repeatability of preconcentration procedure mainly due to the inhomogeneous features of graphene suspension. Therefore, in order to improve homogeneity of graphene suspension and in consequence precision of developed procedure, the non-ionic surfactant, i.e. Triton-X-100, was applied. The experiments show that the surfactant significantly improved repeatability of the sorption process. These effects will be thoroughly discussed in Section 3.3. It prompted us to test whether the addition of surfactant only affects sorption repeatability or it also has an impact on recovery of extracted elements. Fig. 1 shows that the quantitative determination of analytes is impossible when graphene/Triton-X-100 mixture is injected to the analyzed solution. Observed increase in recoveries is associated with precipitation of metal hydroxides just like in the case of graphene without APDC. Eventually, three-component system based on graphene/APDC/Triton-X-100 was chosen for subsequent analysis at pH value of 5. Although, Triton-X-100 does not influence on recovery it significantly improves repeatability (see Section 3.3.).

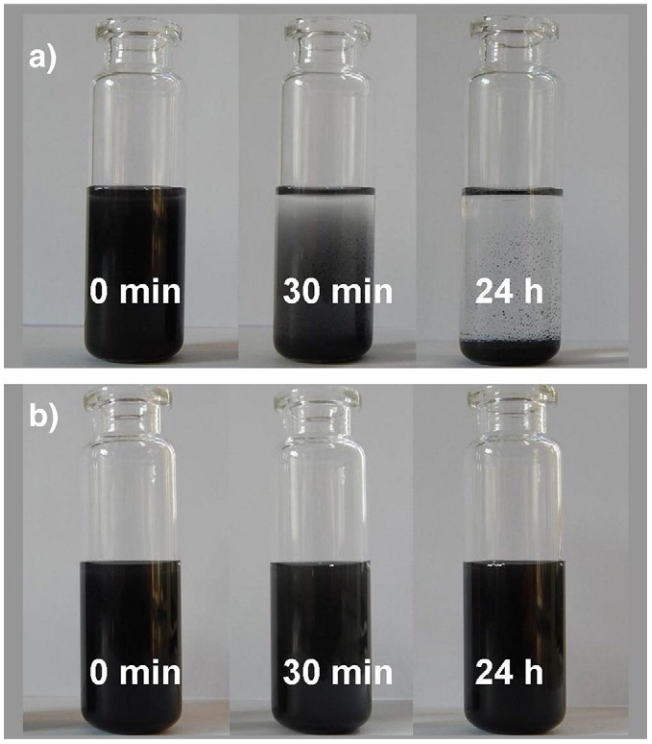

Fig. 4. Suspensions of graphene: a) without surfactant (1 mg mL^{-1} of graphene and 4 mg mL^{-1} of APDC), b) with surfactant (1 mg mL^{-1} of graphene, 0.5 mg mL^{-1} of Triton-X-100 and 4 mg mL^{-1} of APDC).

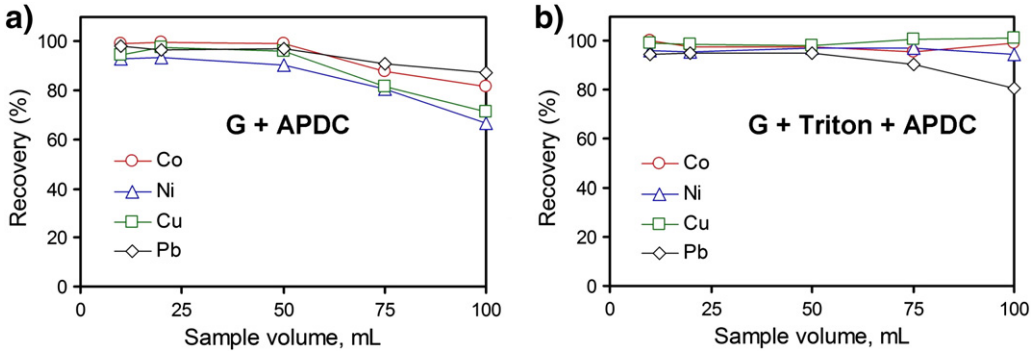

Fig. 5. Effect of sample volume (a) graphene/APDC, (b) graphene/APDC/Triton-X-100 (pH = 5, sample volume 10–100 mL, element concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})); G is the abbreviation for graphene.

3.2. Effect of APDC

According to the fact that sorption process does not occur without the presence of chelating agent (see Fig. 1), the addition of this reagent to the analyzed solution is necessary. The dispensable role of APDC is the formation of hydrophobic complexes which in that form are adsorbed on the graphene surface. Hence, the relationship between APDC concentration and recovery of extracted elements was examined at the reagent concentration ranging from 0 to 2 mg in 50 mL of the sample. The results illustrated in Fig. 2 indicate that the recovery of extracted elements enhanced when APDC amount increased to 0.4 mg and then remained constant. In order to obtain satisfactory recovery and precision, 0.8 mg of APDC was chosen for subsequent analysis.

3.3. Effect of surfactant

As has been already mentioned the main problem encountered during the procedure development resulted from graphene hydrophobic properties. According to the literature, surfactants are employed for improvement of this significant feature which is especially important when graphene suspension is added to the analyzed sample [28,29]. A series of experiments with Triton-X-100 in concentrations ranging from 0 to 0.5 mg mL^{-1} in 200 μL of the mixture containing 1 mg mL^{-1} of graphene and 4 mg mL^{-1} of APDC were performed. RSDs demonstrated in Fig. 3 reveal that the assistance of non-ionic surfactant Triton-X-100 is useful in applied procedure. In the case of Co(II) and Pb(II) RSDs decreased from 10 to 2.5%, when concentration of Triton-X-100 increased to 0.5 mg mL^{-1}. For Ni(II) and Cu(II) RSD values decreased to 2.5% at the lowest examined concentration of Triton-X-100 equaled 0.1 mg mL^{-1} in 200 μL of injected mixture. For the purposes of multielemental analysis Triton-X-100 concentration of 0.5 mg mL^{-1} was chosen for subsequent experiment. In that conditions RSD values are low for all elements and graphene forms stable and homogenous suspension even up to 24 h as it is illustrated in Fig. 4.

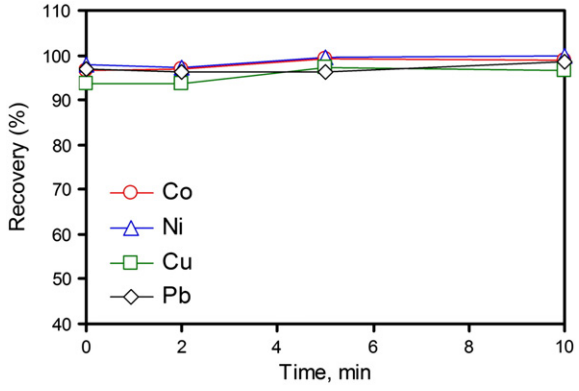

Fig. 6. Effect of sorption time (pH = 5, sample volume 50 mL, element concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})).

3.4. Effect of sample volume

The volume of analyzed solution is a very important parameter, especially when dealing with determination of trace concentration of analytes. The larger volume of the sample is, the higher preconcentration factors and lower DLs can be obtained. On the other hand, sample volume should not be too large — it prolongs filtration step and makes procedure more time consuming. To emphasize the relationship between sample volume and presence of surfactant in analyzed solution, two different combinations of reagents were added to the sample: (i) graphene/APDC and (ii) graphene/APDC/Triton-X-100. In order to evaluate the effect of analyzed solution volume, five different sample volumes (10, 25, 50, 75 and 100 mL) were examined. As can be seen in Fig. 5a, when graphene/APDC mixture is injected to the analyzed solution efficiency of the sorption process decreases over sample volume of 50 mL. It can be explained by the limited contact between solution and adsorbent due to the aggregation of graphene nanosheets. In contrast, when Triton-X-100 solution is added to the analyzed sample (Fig. 5b), recoveries remain on the satisfactory levels for the whole range of examined volumes. We can presume that employment of Triton-X-100 allows homogenous distribution of graphene nanoparticles and prevents them from aggregation. Taking into account that determination of trace amounts of elements requires relatively large sample volume and in the view of further multielemental analysis performance, sample volume of 50 mL was chosen for subsequent step.

3.5. Effect of adsorption time

Time in DMSPE technique is defined as a period between injection of the sorbent and sample centrifugation or filtration. In order to examine influence of contact time on recovery and precision, sorption time was studied within the time range of 0–10 min. Results depicted in Fig. 6 show that sorption of Co(II), Ni(II), Cu(II) and Pb(II) ions is independent in the entire examined time range. This can be explained by the fact that rapid injection of graphene/APDC/Triton-X-100 suspension promotes instant interaction between metal-APDC chelates and graphene nanosheets dispersed throughout the sample. For that reason equilibrium state of that process is achieved quickly and sorption of enriched elements occurs immediately. In order to simplify and shorten developed DMSPE–EDXRF procedure, analyzed solution was immediately filtered after injection of graphene/APDC/Triton-X-100 mixture.

Table 1
The influence of foreign ions on the recovery of determined elements (pH = 5, sample volume 50 mL, analyte concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})); n = 3; uncertainties correspond to one standard deviation.

Matrix element	Matrix element concentration, mg mL^{-1}	Matrix/analyte ratio	Analyte			
			Co	Ni	Cu	Pb
Na^+	10	250 000	101 ± 2.7	98.9 ± 0.4	103 ± 2.1	98 ± 3.5
K^+	0.5	12 500	97 ± 2.0	98 ± 3.6	96 ± 1.5	99 ± 4.1
Ca^{2+}	0.5	12 500	103 ± 1.9	99 ± 2.1	100.0 ± 0.5	101 ± 2.7
Mg^{2+}	1.0	25 000	100 ± 1.4	98.5 ± 0.4	99 ± 1.9	92 ± 2.1
	0.5	12 500	96 ± 3.6	94 ± 2.0	102 ± 1.7	97 ± 4.0
NO_3^-	25	625 000	101 ± 2.7	98.9 ± 0.4	103 ± 2.1	98 ± 3.5
Cl^-	20	500 000	97 ± 2.4	102.7 ± 0.1	102 ± 3.9	100 ± 4.8
SO_4^{2-}	5.0	125 000	97 ± 1.2	101 ± 2.0	99 ± 2.8	92 ± 3.1
	2.0	50 000	100 ± 3.4	97 ± 2.7	100 ± 2.0	98 ± 5.1
HCO_3^-	1.0	25 000	90 ± 1.7	102.9 ± 0.4	103 ± 2.1	94 ± 2.3
	0.5	12 500	98 ± 2.0	97 ± 2.0	96 ± 3.2	100 ± 4.0

3.6. Effect of sample matrix

The effect of coexisting ions that can be found commonly in water samples was investigated. In performed experiments 50 mL of the sample containing 40 ng mL^{-1} of determined elements and an appropriate amount of interfering ions were preconcentrated using developed DMSPE procedure. Alkaline metals i.e. Na(I), K(I), Ca(II) and Mg(II) were introduced to the sample in the form of nitrates. Results listed in Table 1 show that the presence of alkaline metals has no influence on recoveries of enriched elements and recoveries are never worse than 90%, even for relatively high Na(I) concentration. Moreover, an effect of addition of some common anions, i.e. Cl^-, SO_4^{2-}, HCO_3^- and NO_3^- was studied. It was found that the greatest impact on recovery has HCO_3^- ions. Nevertheless, also in this case recoveries have not decreased below 90%. Procedure developed in this work is more resistant to presence of different coexisting ions in comparison with our previous studies where CNTs and GO were applied as solid sorbents [21,25]. It results from the usage of chelating agent with graphene nanosheets characterized by very high adsorption capacity. For example, the maximum contaminant level of Na(I) is 25–60 times higher than those obtained in abovementioned papers. Therefore, the high salinity waters, i.e. sea water, can be analyzed by the developed procedure (Section 3.8).

3.7. Analytical figures of merit of the DSPME procedure

For developed DMSPE–EDXRF procedure the linear ranges, correlation coefficients, sensitivities, recoveries, precision, DLs and enrichment factors were calculated and obtained results are listed in detail in Table 2. To examine linearity of proposed procedure a series of multielemental solutions containing Co(II), Ni(II), Cu(II) and Pb(II) in concentrations of 0, 5, 10, 20, 50, 75 and 100 ng mL^{-1} were prepared. The results show that proposed analytical procedure is linear in the entire range of examined concentrations, which is confirmed by good correlation coefficients varying from 0.9997 for Co(II) to 0.9970 for Pb(II).

It is noteworthy that the samples are analyzed as a thin layer (200 μg of graphene collected onto membrane filter of 5 mm in diameter). Therefore, the matrix effects can be neglected and, in consequence, the linear relationship between radiation intensity and analyte concentration is obtained [30]. The recovery values for each examined element are high and range from 96% in the case of Ni(II) and Pb(II), to 97% for Co(II) and 99% for Cu(II) ions. As it was mentioned above, developed DMSPE–EDXRF methodology is greatly simplified by reducing number of operations. Thus, the number of errors that may occur during the procedure performance is greatly reduced and obtained RSD values are low (for Co(II), Ni(II), Pb(II) ca. 2.6–2.8% and for Cu(II) 3.4%). Another important factor characterizing analytical procedure is sensitivity of the developed methodology. In Fig. 7 two different EDXRF spectra are presented: the one obtained for direct analysis of aqueous standard solution containing 50 μg mL^{-1} of analyzed elements (blue spectrum) and another one obtained after preconcentrating the solution containing 50 ng mL^{-1} of determined elements (red spectrum). Although concentration of determined elements in the second situation is 1000 times lower, much better signal-to-background ratio is observed which results from the excellent sensitivity of the proposed methodology. Enrichment factors obtained with the use of described sorption procedure equaled ca. 2200–2500 for Co(II), Ni(II) and Cu(II), and ca. 400 for Pb(II). It should be mentioned here, that enrichment factors were calculated as ratio of sensitivity of DMSPE–EDXRF procedure to sensitivity of direct EDXRF analysis. The high enrichment factor values allow low DLs to be obtained, ca. 10,000 times lower than DLs received by direct EDXRF analysis. To obtain the best DLs, an evaluation of different measuring

Table 2
Analytical figures of merit of DMSPE/EDXRF procedure (laboratory-constructed EDXRF spectrometer, 5 mm collimator, Rh target X-ray tube operated 40 kV and 0.300 mA); uncertainties correspond to one standard deviation.

	Co	Ni	Cu	Pb
Recovery (%) (n = 10)	97 ± 2.6	96 ± 2.5	99 ± 3.4	96 ± 2.7
RSD (%)	2.7	2.6	3.4	2.8
Sensitivity, mL ng^{-1} s^{-1} mA^{-1}	9.1 ± 0.10	8.3 ± 0.10	8.4 ± 0.12	2.23 ± 0.086
Enrichment factor	2242 ± 25	2440 ± 30	2553 ± 36	418 ± 16
DL, ng mL^{-1} (300 s)	0.33	0.47	0.42	1.5
DL, ng mL^{-1} (600 s)	0.23	0.34	0.29	1.1
Maximum concentration, ng mL^{-1}	100	100	100	100
Correlation coefficients	0.9997	0.9996	0.9996	0.9970

The detection limits were calculated from equation DL = $(3/k)(R_B/t)^{1/2}$, where k is the sensitivity of the method, R_B is the background count rate in counts s^{-1} and t is the counting time.

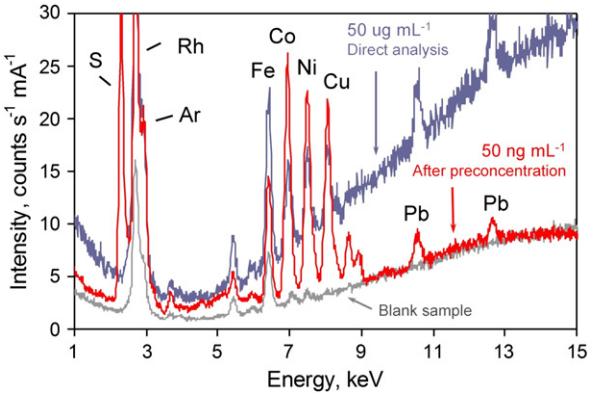

Fig. 7. Comparison between EDXRF spectra obtained for the direct analysis of an aqueous standard solution containing 50 μg mL^{-1} of determined elements (blue line), after preconcentration of sample containing 50 ng mL^{-1} of these elements using DMSPE–EDXRF procedure (red line) and blank sample (gray line).

modes (kV/mA/primary beam filters) was carried out. The current of the X-ray tube was fixed to obtain dead-time loss of less than 50%. As can be seen in Table 3, the best DLs for Co, Ni, Cu can be obtained for 30 kV/0.100 mA/none-filter and 30 kV/0.300 mA/100 μm Ag. In case of Pb, much better DL was obtained for 30 kV/0.300 mA/100 μm Ag. It results from very low background achieved for 100 μm Ag primary beam filter. It is noteworthy here that obtained DLs are from 2 to 25 times lower than DLs received in our previous research (CNTs or GO as solid sorbents) even if polarized EDXRF spectrometry was applied [21–24]. It results from the possibility of using small amount of the solid sorbent and in consequence obtaining thin samples of small diameter. In this way, due to the low scattering of continuum spectrum of X-ray tube the excellent signal to background ratio is achieved. It is worth mentioning that obtained DLs are below the maximum contaminant levels for each of determined element that might be found in drinking water according to EPA regulations (15 ng mL^{-1} of Pb(II) [31], 100 ng mL^{-1} of Ni(II) [32] and 1300 ng mL^{-1} of Cu(II) [33]).

3.8. Analytical application

The proposed DMSPE–EDXRF procedure was successfully applied for the determination of trace amounts of Co(II), Ni(II), Cu(II) and Pb(II) ions in different water samples e.g. tap water, lake water and mineral water. Taking into account that developed methodology is perfect for analysis of waters containing large amounts of salts, synthetic sea water sample was examined as well. The reliability of the proposed methodology was tested with the use of a sample spiked with a known concentration of each determined element. Analyzed water samples were spiked with 20 and 50 ng mL^{-1} of Co(II), Ni(II), Cu(II) and Pb(II) ions. The results listed in Table 4 show that the recoveries of enriched elements are reasonable for trace analysis and averaged 104, 103, 99 and 100% for Co(II), Ni(II), Cu(II) and Pb(II) ions, respectively.

4. Conclusions

The proposed procedure is based on DMSPE using graphene as a solid sorbent. This approach eliminates problems occurring commonly when graphene nanosheets are applied in classical SPE. In DMSPE, graphene nanosheets are dispersed in aqueous samples, which promote immediate interaction between the metal chelates and graphene, thus the preconcentration procedure is very fast and sorption process is almost time independent. Moreover, some of the graphene properties such as high surface area, high adsorption capacity and good dispersibility supported by the surfactant addition make graphene an ideal sorbent for DMSPE. In the proposed procedure, DMSPE is combined with direct measurement of metal ions adsorbed on the graphene surface using the EDXRF technique. Therefore, an additional elution of analytes is not required, in contrast to other spectroscopy techniques. In consequence, the number of chemicals required to perform an experiment and the number of performed operations are significantly reduced. According to the fact that both sides of graphene planar sheets participate in sorption process, the amount of solid sorbent required to achieve quantitative recovery is greatly reduced. It enables us to obtain thin samples of small diameter, characterized by a high signal to background ratio, which results directly in decrease of obtained DLs. All of the abovementioned qualities contribute to great suitability of graphene in EDXRF measurement. It is also caused by the fact that graphene nanosheets do not emit X-ray characteristic radiation. Moreover, the particle size effects can be neglected due to very small size of graphene sheets. Summing up, the developed DMSPE–EDXRF combination with graphene as a sorbent enables low DLs to be obtained in a very simple, fast, inexpensive and environmental friendly way.

Table 3
The detection limits obtained for various measurement conditions (benchtop EDXRF spectrometer — Epsilon 3, Rh target X-ray tube, counting time 300 s).

Voltage, kV	Current, mA	Primary beam filter	DL, ng mL^{-1}			
			Co	Ni	Cu	Pb
30	0.100	None	0.092	0.10	0.079	1.1
20	0.170	None	0.10	0.10	0.13	1.2
30	0.300	100 μm Ag	0.080	0.073	0.080	0.20
20	0.450	100 μm Ag	0.17	0.13	0.17	0.83
30	0.300	7 μm Ti	0.17	0.25	0.21	0.35
20	0.450	7 μm Ti	0.37	0.56	0.45	0.55

Table 4
Determination of Co(II), Ni(II), Cu(II) and Pb(II) in spiked water samples (pH = 5, sample volume 50 mL, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (4 mg mL^{-1}) and Triton-X-100 (0.5 mg mL^{-1})); n = 3; uncertainties correspond to one standard deviation.

Sample	Element	Added, ng mL^{-1}	Found, ng mL^{-1}	Recovery (%)
Tap water	Co	0	<DL	
		20	20.3 ± 0.27	101.4
		50	52.0 ± 0.74	104.1
	Ni	0	<DL	
		20	21.8 ± 0.96	108.9
		50	48.7 ± 0.29	
	Cu	0	8.8 ± 0.70	
		20	28 ± 1.2	96
		50	59.5 ± 0.21	101.5
	Pb	0	<DL	
		20	19.3 ± 0.10	96.4
		50	55 ± 1.9	110
Lake water	Co	0	<DL	
		20	22.0 ± 0.68	109.6
		50	52.1 ± 0.10	104.0
	Ni	0	5.3 ± 0.28	
		20	25 ± 1.0	99
		50	55.6 ± 0.37	100.4
	Cu	0	4.5 ± 0.57	
		20	24 ± 1.3	98
		50	52 ± 2.9	95
	Pb	0	6 ± 1.7	
		20	24.7 ± 0.58	94
		50	59 ± 2.2	106
Mineral water	Co	0	<DL	
		20	21.0 ± 0.39	104.9
		50	53.9 ± 0.60	107.8
	Ni	0	<DL	
		20	20.1 ± 0.47	100.3
		50	51.2 ± 0.85	102.4
	Cu	0	6.8 ± 0.31	
		20	26 ± 2.0	96
		50	59 ± 3.4	104
	Pb	0	<DL	
		20	20 ± 1.3	100
		50	49 ± 3.2	98
Synthetic sea water	Co	0	4.4 ± 0.10	
		20	23.6 ± 0.92	95.9
		50	55.6 ± 0.32	102.4
	Ni	0	<DL	
		20	21.2 ± 0.10	105.8
		50	51.4 ± 0.26	102.7
	Cu	0	14 ± 1.0	
		20	33 ± 1.9	95
		50	66 ± 2.6	104
	Pb	0	<DL	
		20	19 ± 1.6	95
		50	52 ± 1.3	104

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Determination and speciation of trace and ultratrace selenium ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid adsorbent in dispersive micro-solid phase extraction

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ABSTRACT

A dispersive micro-solid phase extraction (DMSPE) with graphene as a solid adsorbent and ammonium pyrrolidinedithiocarbamate (APDC) as a chelating agent was proposed for speciation and detemination of inorganic selenium by the energy-dispersive X-ray fluorescence spectrometry (EDXRF). In developed DMSPE, graphene particles are dispersed throughout the analyzed solution, therefore reaction between Se(IV)–APDC complexes and graphene nanoparticles occurs immediately. The concentration of Se(VI) is calculated as the difference between the concentration of selenite after and before prereduction of selenate. A central composite face-centered design with 3 center points was performed in order to optimize conditions and to study the effect of four variables (pH of the sample, concentration of APDC, concentration of Triton-X-100, and sample volume). The best results were obtained when suspension consisting of 200 μg of graphene nanosheets, 1.2 mg of APDC and 0.06 mg of Triton-X-100 was rapidly injected to the 50 mL of the analyzed solution. Under optimized conditions Se ions can be determined with a very good recovery (97.7 ± 5.0% and 99.2 ± 6.6% for Se(IV) and Se(VI), respectively) and precision (RSD = 5.1–6.6%). Proposed DMSPE/EDXRF procedure allowed to obtain low detection limits (0.032 ng mL^{-1}) and high enrichment factor (1013 ± 15). The proposed methodology was successfully applied for the determination of Se in mineral, tap, lake and sea water samples as well as in biological materials (Lobster Hepatopancreas and Pig Kidney).

1. Introduction

Selenium is an essential micronutrient, necessary for the proper functioning of human and animal organisms due to its antioxidant properties and presence in selenoproteins [1]. Se captures, degrades and inhibits the formation of free radicals which protects cells from damage, metabolic disorders and premature aging [2]. Recently, large international projects are conducted in order to investigate the beneficial effect of Se on diseases such as AIDS, diabetes or cancer [3]. The daily requirement for adults ranges from 50 to 70 μg [4], however the difference between the toxic dose and the nutritious requirement is very small and exceeding the tolerable upper intake level of 400 μg per day may lead to selenosis [5]. Se can be found most frequently in natural waters as organic selenides (-II) or inorganic selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) [6]. The toxicity of inorganic Se forms is up to 40 times higher than the organic ones [7], wherein selenates are more toxic than selenites [8]. For that matter, the development of the reliable analytical procedures enabling not only determination of total Se amount but also speciation analysis is a subject of a great importance.

The most popular analytical techniques applied for inorganic Se determination are hydride generation coupled with the atomic absorption spectrometry (AAS) [9,10], inductively coupled plasma atomic emission spectroscopy (ICP-OES) [11,12], and atomic fluorescence spectroscopy (AFS) [13,14]. Nevertheless, the direct determination of Se species in food, biological or environmental samples is usually hampered by its low concentration. Therefore, preconcentration step is usually required prior to quantitative analysis in order to improve sensitivity and precision of applied techniques. This approach is also very useful when the influence of complicated matrix should be reduced.

Different preconcentration procedures, i.e. co-precipitation with hydroxides [15], extraction [16], microextraction [17–22], anion exchange chromatography [23] and isotachophoresis [24] have been recently proposed for Se determination. Despite unquestionable advantages of the proposed methodologies, the most frequently employed procedures for Se preconcentration are based on the solid

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phase extraction (SPE). Analytical methods for the speciation and preconcentration of inorganic Se by SPE have been recently reviewed [25]. SPE is an inexpensive alternative to classical liquid-liquid extraction approach. It allows obtaining cleaner extracts, higher and more reproducible recoveries and can be also readily automated. Furthermore, SPE simplifies selective adsorption of analytes due to numerous sorbent materials and solvent mixtures that can be employed in this technique. Three different SPE strategies can be applied in order to perform speciation analysis of Se. The first one involves sorption of desired Se form while the other Se species are not retained. In the next step the captured analyte is eluted by an appropriate solvent. The second procedure bases on simultaneous sorption of selanites and selenates on the SPE adsorbent surface and subsequent elution of both species or just one Se form. In the case of simultaneous elution of both inorganic Se forms posterior speciation involving capillary electrophoresis or liquid chromatography is usually required. The last strategy involves chelates formation and subsequent sorption of complexed species. The most commonly used complexing agents are selective for only one form of Se, the most frequent Se(IV). Numerous adsorbents were successfully used in abovementioned strategies for Se determination purposes, i.e. iron(III) loaded Chelex®-100 [26], Amberlite XAD-4 grafted with DAN [27], mercapto-silica [28], activated carbon [29], Al_2O_3 [30], TiO_2 [31], Mo-form-resin [32], (CTAB)-modified alkyl silica [33], Dowex 1×2 [34]. Another types of adsorbents used for preconcentration of Se and other metal ions are nanoparticles such as nanosized-Al_2O_3 [35,36] and TiO_2 [37,38] as well as carbon-based nanomaterials such as carbon nanotubes [39,40], graphene [41,42] and graphene oxide [43,44]. Nowadays graphene is becoming more and more popular as an adsorbent due to its unique physicochemical properties [45,46]. Graphene consists of sp^2-hybridized carbon atoms organized in the honeycombed sheets providing great surface area and adsorption capacity. It is noteworthy that in contrast to carbon nanotubes both sides of planar layers are involved in adsorption process. Moreover, such a structure promotes formation of π–π stacking interaction with carbon-based ring structures.

In the present work a dispersive micro-solid phase extraction (DMSPE) with graphene as a solid adsorbent and ammonium pyrrolidinedithiocarbamate (APDC) as a chelating agent was applied for determination of trace and ultratrace Se ions by energy-dispersive X-ray fluorescence spectrometry (EDXRF). In the DMSPE a small amount of solid adsorbent is dispersed in the analyzed solution. This approach eliminates different problems that may occur in classical SPE mode such as nano-sorbent particles escaping from the cartridges or formation of high pressure inside the SPE system. Application of EDXRF spectrometry eliminates necessity of additional elution of analytes, since samples can be measured in the form of thin layers deposited onto the membrane filters.

2. Experimental

2.1. Chemicals and solutions

Stock solution (1 mg mL^{-1}) of Se(IV), nitric acid (65%, Suprapur®) and ammonium hydroxide solution (25%, Suprapur®) were purchased from Merck (Darmstadt, Germany). Hydrochloric acid (35–38%, p.a.), Triton-X-100 and reagents applied for examination of matrix elements influence, i.e. $NaNO_3$, KNO_3, $Ca(NO_3)_2$, $Mg(NO_3)_2$, NaCl, Na_2SO_4, $NaHCO_3$ were purchased from POCh (Gliwice, Poland). Standard solutions of 10 μg mL^{-1} of Se(IV) were prepared from the stock solution. 10 μg mL^{-1} of Se(VI) standard solution was prepared by dissolving in water proper amount of sodium selenate – Na_2SeO_4 (≥98%) purchased from Sigma-Aldrich (St. Louise, MO, USA). Graphene of 8 nm thickness, ammonium pyrrolidine dithiocarbamate – APDC (>98%) were purchased from Graphene Supermarket® (Calverton, USA) and Acros Organics (Geel, Belgium), respectively. A high purity water obtained from the Milli-Q system was used for solutions preparation.

2.2. Instrumentation

The measurements were performed using an EDXRF Epsilon 3 spectrometer (Panalytical, Almelo, The Netherlands), equipped with Rh target X-ray tube of a 50 μm Be window and a max. power of 9 W. The X-ray spectra were collected using a thermoelectrically cooled silicon drift detector (SDD) with 8 μm Be window and 135 eV resolution at 5.9 keV. The system is also equipped with 10-position removable sample changer, spinner and five primary filters that can be selected to improve measuring conditions for determined element. Evaluation of spectra was performed using non-linear least squares fitting, based on the AXIL algorithm (Epsilon 3 software).

2.3. Preconcentration procedure

The suspension of graphene/APDC/Triton-X-100 was prepared by dissolving: 10 mg of graphene, 60 mg of APDC and 3 mg of Triton-X-100 in 10 mL of high purity water. Subsequently, the mixture was sonicated for 60 min in order to obtain homogenous graphene suspension. Additionally, graphene/APDC/Triton-X-100 mixture was sonicated for 5 min before application. The rapid injection of 200 μL of graphene/APDC/Triton-X-100 suspension to 50 mL of analyzed solution was preceded by an adjustment of pH value to 1. In the next step, sample was filtered through the membrane filter with the use of filtration assembly of 5 mm in diameter. Finally, the membrane filter with graphene and Se(IV)–APDC complex adsorbed on its surface was dried under an IR heater and measured using EDXRF spectrometry.

2.4. Sample preparation

2.4.1. Water samples

All examined water samples were filtered through a Millipore cellulose acetate membrane filters (0.45 μm) and stored at 4 °C. In order to prepare an artificial sea water solution 21.03 g NaCl, 3.52 g Na_2SO_4, 0.61 g KCl, 0.088 g KBr, 0.034 g $Na_2B_4O_7\cdot10H_2O$, 9.50 g $MgCl_2\cdot6H_2O$, 1.32 g $CaCl_2\cdot2H_2O$, 0.02 g $SrCl_2\cdot6H_2O$ and 0.02 g $NaHCO_3$ was dissolved in 1 L of high purity water [47].

2.4.2. Biological samples

200 mg of Lobster Hepatopancreas Certified Material (TORT-2) and 100 mg of Pig Kidney (ERM®-BB186) were digested in 5 mL of concentrated HNO_3 using microwave-assisted digestion (closed 100 mL vassels; pressure set to 45 atm). Subsequently, digested samples were quantitatively transferred into 100 mL beakers and evaporated. Finally, 50 mL of high purity water was added to the residue and preconcentration procedure described in Section 2.3. was performed.

3. Results and discussion

3.1. Optimization of preconcentration procedure

Central composite design (CCD) was applied in order to achieve maximal information about DMSPE/EDXRF proconcentration procedure from a limited number of experiments. A face-centered central composite design (FCCCD) is one of designs commonly applied for estimating the coefficients of a model containing a

constant, the linear terms, the interactions between pairs of variables and the quadratic terms. It is obtained by combining a factorial design at two levels with a star design and n center points, and each variable is studied at three equally spaced levels, coded as -1, 0 and $+1$.

In order to optimize the conditions and to study the effect of four variables (pH of the sample, concentration of APDC, concentration of Triton-X-100, and sample volume) a FCCCD has been performed. Each of the 24 experiments of the design ($4^2+4\times2$) was run in duplicate, while the center point has been replicated 6 times. The domain of pH was set between 1 and 5 relying on experiments in which APDC was applied as a chelating agent for Se ions [48–51]. The other variables under study and their ranges (0.4, 1.2 and 2 mg of APDC; 0.02, 0.06 and 0.1 mg of Triton-X-10; and sample volume of 50, 75 and 100 mL) were determined from preliminary experiments. The variables and values used for FCCCD are listed in Table S1 in Supplementary materials. The amount of graphene (0.2 mg) was constant in all experiments. This first set of experiments (data not reported) showed very clearly the strong effect of pH, with the worst experiment at pH 1 giving a response of 96.1%, the worst experiment at pH 3 having a response of 88.3% and the best experiment at pH 5 producing a response of 18.1%. As a consequence of this, it has been decided to perform a new FCCD with three center points in which the pH was kept constant at 1 and the remaining variables had the same domain. Table S2 included in Supplementary materials shows the experimental matrix and the responses obtained (the experiments were performed in random order). By looking at the data table, it can be seen that the results are very similar for all the experiments, being in the range 97.0–103.6, with the three replicates of the center point being in the range 97.5–100.0. The standard deviation computed on the 17 experiments is 1.9, while the standard deviation of the three replicates is 1.3. The F test ($F_{exp}=2.21$, $F_{0.95,16,2}=19.43$) shows that the variance of the replicates is not significantly larger than the variance of the whole matrix. This means that the variability induced by modifying the experimental conditions inside the experimental domain is not larger than the pure experimental variability. Therefore, it can be said that the three variables under study have no effect. The final conditions chosen for further analysis are: pH=1, 50 mL of sample and 200 μL of suspension of graphene/APDC/Triton-X-100 (1 mg mL^{-1} of graphene, 6 mg mL^{-1} of APDC and 0.3 mg mL^{-1} of Triton-X-100), i.e. containing 0.2 mg of graphene, 1.2 mg of APDC and 0.06 mg of Triton-X-100. It is worth noting here that the sample volume have no influence on Se recovery in the studied range. Therefore, the analysis of real samples can be performed using the sample volume from 50 to 100 mL to obtain adequate enrichment factor and in consequence detection limits fitted for the purpose.

3.2. The effect of matrix elements

The effect of different cations and anions commonly present in waters was investigated. Maximum concentrations of foreign elements and its influence on recovery of Se are listed in Table S3 in Supplementary materials. Neither the alkaline metals nor some common ions present in water samples have influence on recovery of Se, which in every case is higher than 98%. DMSPE/EDXRF procedure is characterized by an excellent resistance to presence of coexisting ions which is especially exposed for Na^+, Cl^-, SO_4^{2-} and NO_3^-, where matrix to analyte ratio equals or exceeds 250,000. Mg^{2+} and HCO_3^- ions have the greatest impact on recovery of selenium, however obtained recoveries are still at high level (>98%) for relatively high excess of coexisting ions. This great resistance can be explained by the fact that alkali and alkaline earth elements do not react with APDC, however large amount of this ions appreciably reduce the extraction efficiency.

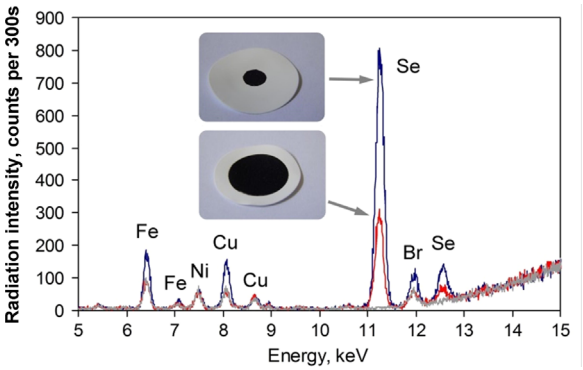

Fig. 1. Comparison between EDXRF spectra obtained for the two samples of different diameters – 5 mm (blue line) and 22 mm (red line), excited by an X-ray beam of a 5.9 mm of focal spot size. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Analytical figures of merit of DMSPE procedure

The thickness and the diameter of obtained samples play very significant role for the purposes of XRF measurement. Proposed DMSPE/EDXRF procedure enables to obtain samples in form of thin layers, since small amount of adsorbent is necessary to perform the quantitative recovery of Se ions. For that matter, errors resulting from matrix effects can be neglected. The benchtop EDXRF spectrometers equipped with low-power X-ray tube usually gives X-ray beam of a relatively small spot size. Therefore, the best sensitivity can be obtained for the samples collected on the small area. In other words, if the area of the specimen is too large, only a small fraction of the preconcentrated element is excited and as a consequence a low intensity of fluorescent radiation is observed. Fig. 1 presents the EDXRF spectra of two samples of different diameters (5 and 22 mm; mass per unit area equals 1020 and 53 μg cm^{-2}, respectively) excited by an X-ray beam of 5.9 mm focal spot size calculated as full width at half maximum. It is noteworthy that 22 mm is the diameter of the sample obtained with the use of commercially available filtration assembly of 25 mm. As can be observed, when the diameter of the sample is fitted to small spot size of incident X-ray beam, a high intensity of fluorescent radiation is observed. It should be noted here that although the specimen diameter is small the specimen forms thin layer. Such thin specimen of small diameter can be obtained for only small amount of graphene (200 μg) that is sufficient for quantitative adsorption of Se ions. In consequence, the matrix effects in thin layer can be neglected and the linear relationship between radiation intensity and Se concentration is obtained. In order to obtain satisfactory detection limits and the best sensitivity, samples of 5 and 22 mm diameter were measured with the use of different measuring modes (voltage, current and beam filters). The detection limits were calculated from equation $DL=(3/k)(R_B/t)^{1/2}$, where k is the sensitivity of the method, R_B is the background count rate in counts s^{-1} and t is the counting time. The results in Table 1 show that 5 mm samples present 3.2–3.5 times better sensitivity than 22 mm samples for all examined measurement conditions. It results in very good signal to background ratio and as a consequence very low DLs values are observed for samples of smaller diameter. Generally, the lowest DLs were achieved for 5 mm samples and 100 μm Ag filter (0.032 ng mL^{-1}) and these conditions were chosen for the subsequent experiment.

Analytical figures of merit of DMSPE/EDXRF procedure for optimized preconcentration and measurement conditions are presented in Table 2. Obtained results show that the procedure is linear in the entire range of examined concentrations (up to 500 ng mL^{-1}) which reflects in a very good correlation coefficient. The recovery

Table 1
Measurement conditions, sensitivities and DLs obtained using the optimized DMSPE/EDXRF procedure.

Voltage (kV)	Current (mA)	Primary beam filter	DL (ng mL^{-1})		Sensitivity (mL ng^{-1} s^{-1})	
			5 mm	22 mm	5 mm	22 mm
30	75	None	0.15	0.40	29.6	9.31
30	300	100 μm Ag	0.032	0.088	8.07	2.33
30	300	7 μm Ti	0.11	0.21	118	33.8
30	300	200 μm Al	0.058	0.18	88	24.9
30	300	50 μm Al	0.065	0.22	109	34.3

Table 2
The parameters characterizing DMSPE/EDXRF procedure.

	Se(IV)	Se(VI)
Recovery (%) ($n=10$)	97.7 ± 5.0	99.2 ± 6.6
RSD (%)	5.1	6.6
Sensitivity, mL ng^{-1} s^{-1} (100 μm Ag)	8.07	
DL, ng mL^{-1} (100 μm Ag)	0.032	
Enrichment factor	1013 ± 15	
Maximum concentration, ng mL^{-1}	500	
Correlation coefficients	0.9995	

values determined both for Se(IV) and Se(VI) are high and equal to 98 and 99%, respectively. Developed DMSPE/EDXRF methodology is characterized by a very good precision due to reduced number of operations and errors that may occur during the procedure performance. RSDs equal 5.1 and 6.6% for Se(IV) and Se(VI), respectively. It is worth to emphasize that according to the Polish regulations of natural mineral water, branch water and bottled water, the precision and the accuracy of the method for Se determination should not be worse than 10% [52]. It is also noteworthy here that obtained DLs are below maximum contaminant levels of drinking water established by the Polish regulations for bottled water (10 ng mL^{-1}) [52] and EPA (50 ng mL^{-1}) [53]. Such excellent DL could be obtained due to the high enrichment factor (calculated as ratio of sensitivity of DMSPE/EDXRF procedure to sensitivity of direct EDXRF analysis of liquid sample).

3.4. Analytical application

The reliability of proposed methodology was verified using spiked samples of tap, lake and sea water. The results listed in Table 3 show that the relative Se recoveries from these water samples were in the range of 96–106%. Obtained results show that no matrix effect was observed. The proposed methodology was also applied for speciation analysis of Se. Just as in the case of real water samples analysis, analyzed samples were spiked with known amount of proper Se species (10 ng mL^{-1}). Since APDC forms complexes only with Se(IV) ions, concentration of that specimen was determined with the DMSPE/EDXRF procedure. The sum of Se(IV) and Se(VI) was determined after reduction of Se(VI) to Se(IV) using reduction procedure described in Ref. 22. The concentration of Se(VI) was calculated as the difference between total amount of Se and Se(IV) concentrations. Result listed in Table 4 indicates that proposed methodology can be successfully applied for determination of Se in real water samples with good recovery and precision. The reliability of proposed methodology was also examined by an analysis of two reference materials – Lobster Hepatopancreas (TORT-2) and Pig Kidney (ERM®-BB186). Results presented in Table 5 show excellent agreement between certified and reference concentration of Se ions.

Table 3
Determination of Se in spiked water samples. (pH = 1, sample volume 50 mL, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (6 mg mL^{-1}) and Triton-X-100 (0.3 mg mL^{-1})); $n=3$; uncertainties correspond to one standard deviation.

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)
Lake water	0	< DL	
	1	1.02 ± 0.06	102.0
	5	4.8 ± 0.27	96.0
	10	10.1 ± 0.51	101.0
	20	19.3 ± 0.44	96.5
	50	53.1 ± 1.10	106.2
Synthetic sea water	0	< DL	
	1	1.01 ± 0.06	101.0
	5	4.9 ± 0.16	98.0
	10	10.3 ± 0.21	103.0
	20	21.0 ± 0.32	105.0
	50	48.9 ± 0.84	97.8
Tap water	0	< DL	
	1	1.01 ± 0.06	101.0
	5	4.9 ± 0.16	98.0
	10	10.3 ± 0.21	103.0
	20	19.7 ± 0.52	98.5
	50	51.9 ± 1.33	103.4

Table 4
Determination of Se(IV) and Se(VI) in spiked mineral water samples.

Added (ng mL^{-1})		Found (ng mL^{-1})		Recovery (%)	
Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
0	0	1.6 ± 0.10	< DL	–	–
10.0	0	11.4 ± 0.19	< DL	98	–
0	10.0	< DL	9.9 ± 0.65	–	99
10.0	10.0	11.0 ± 0.36	9.7 ± 0.81	94	97

4. Conclusions

In present work graphene was applied for the first time for speciation and determination of inorganic Se in different water and biological samples. Although graphene is insoluble and hard to disperse in aqueous solutions, application of nonionic surfactant gives the possibility to use it as a solid adsorbent. Due to unique surface area and its hexagonal arrays very small amount of that nanomaterial is necessary to prepare the sample (only 200 μg per 50–100 mL of the sample). Application of DMSPE technique results in shortening of the sample preparation time, since the equilibrium state is achieved immediately and sorption time is almost time independent. In comparison to other preconcentration techniques reported in the literature for Se determination (Table 6), developed DMSPE/EDXRF procedure enables to obtain very low DLs (even better than mass spectrometry) and very good repeatability. Although, in some cases, application of ETAAS methodology allows obtaining lower detection limits, the advantage of EDXRF over ETAAS relies in the non-destructive character of the measurement. Moreover, ETAAS can be considered as an expensive and time consuming measurement technique. Enrichment factor which can be obtained after the application of the proposed procedure is very high (1013 ± 15), even if we compare it to DLLME techniques. Very good precision of DMSPE/EDXRF procedure results from the simplicity of the proposed methodology consisting only from two main steps (dispersion of the graphene suspension in analyzed solution and filtration). Another advantage which results from application of proposed methodology is the possibility of high salinity water analysis, since the combination of DMSPE/EDXRF is characterized by an excellent resistance to presence of coexisting ions.

Table 5
Analytical result of Se determination in certified reference materials with the DMSPE/EDXRF methodology; $n=3$.

Material	Coexisting elements[a] ($\mu g\ g^{-1}$)	Certified concentration of Se ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	Accuracy (%)
TORT-2	As (21.6), Cd (26.7), Cu (106), Fe (105), Mn (13.6), Ni (2.5), Sr (45.2), V (1.64), Zn (180)	5.63 ± 0.67	5.8 ± 0.48	3.0
ERM®-BB186	Cd (1.09), Cu (36.5), Fe (256), Mn (7.26), Zn (134)	10.3 ± 0.9	10.5 ± 0.32	1.9

[a] Elements at concentration below 1 $\mu g\ g^{-1}$ are not included in the Table.

Table 6
Recent applications of different preconcentration procedures for Se determination.

Preconcentration procedure	Sample volume (mL)	DL ($ng\ mL^{-1}$)	RSD (%)	pH	EF[a]	Determine technique	Recovery (%)	Linearity ($ng\ mL^{-1}$)	Ref.
Coprecipitation with La $(OH)_3$	6.8	3	1.2	0.12% (w/v) NaOH	20	FI–GH–AAS[e]	> 94	0.02–1.0	[15]
DLLME[b]	5	2.1	3	5	250	EDXRF	95	up to 400	[18]
DLLME	5	0.05	4.5	3	70	ETAAS[f]	> 96	0.1–3.0	[19]
DLLME	4	0.015	5.1	0.5 mol/L HCl	20	ETAAS	> 96	up to 12.5	[20]
HF-LPME[c]	10	0.005	3.1	4	480	ETAAS	> 94	0.05–35	[21]
CPE[d]	10	0.5	3.5	6	50	ETV–ICP-MS[g]	> 95	up to 50	[16]
DMSPE	50	0.032	5.1–6.6	1	1013	EDXRF	> 98	up to 500	This work

[a] Enrichment factor.
[b] Dispersive liquid–liquid microextraction.
[c] Hollow fiber liquid-phase microextraction.
[d] Cloud point extraction.
[e] Flow injection–hydride generation–atomic absorption spectrometry.
[f] Electrothermal atomic absorption spectrometry.
[g] Electrothermal vaporization–inductively coupled plasma-mass spectrometry.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.11.036.

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Supplementary Materials

Determination and speciation of trace and ultratrace selenium ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid adsorbent in dispersive micro-solid phase extraction

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Table S-1
The variables and values used for FCCCD design

Factor	Level		
	Low (-1)	Medium (0)	High (+1)
pH	1	3	5
APDC, mg	0.4	1.2	2
Sample volume, mL	50	75	100
Triton-X-100, mg	0.02	0.06	0.1

Table S-2
List of experiments in the FCCD and obtained responses

	APDC	Sample volume	Triton-X-100	Recovery(%)
Experiment				
1	1	1	1	100.2
2	1	1	-1	98.6
3	1	-1	1	98.2
4	1	-1	-1	98.7
5	-1	1	1	97.0
6	-1	1	-1	98.5
7	-1	-1	1	98.4
8	-1	-1	-1	103.6
9	-1	0	0	99.5
10	1	0	0	94.9
11	0	-1	0	99.5
12	0	1	0	98.5
13	0	0	-1	96.1
14	0	0	1	100.6
15	0	0	0	99.3
16	0	0	0	100.0
17	0	0	0	97.5

Table S-3
The effect of coexisting ions on the recovery of Se (pH = 1, sample volume 50 mL, analyte concentration 0.04 μg mL^{-1}, 200 μL of suspension containing graphene (1 mg mL^{-1}), APDC (6 mg mL^{-1}) and Triton-X-100 (0.3 mg mL^{-1})); n = 3; uncertainties correspond to one standard deviation

Matrix element	Added salt	Matrix element concentration, mg mL^{-1}	Matrix/ analyte ratio	Recovery (%)
Na^+	$NaNO_3$	10	250 000	98.6 ± 1.7
K^+	KNO_3	0.5	12 500	101.5 ± 2.2
Ca^{2+}	$Ca(NO_3)_2$	0.5	12 500	100.2 ± 3.1
Mg^{2+}	$Mg(NO_3)_2$	1.0	25 000	98.2 ± 2.2
NO_3^-	$NaNO_3$	25	625 000	98.6 ± 1.7
Cl^-	NaCl	20	500 000	100.7 ± 2.5
SO_4^{2-}	Na_2SO_4	5.0	125 000	99.2 ± 3.0
HCO_3^-	$NaHCO_3$	1.0	25 000	98.4 ± 1.9

10. ZAŁĄCZNIKI

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10.2. DOROBEK NAUKOWY

Indeks Hirscha - 4
Liczba cytowań bez autocytowań – 53*

*Według strony Web of Science

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10.3. OŚWIADCZENIA WSPÓŁAUTORÓW

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Oświadczenie

Oświadczam, że w pracy R. Sitko, K. Kocot, B. Zawisza, B. Feist, K. Pytlakowska, Liquid-phase microextraction as an attractive tool for multielement trace analysis in combination with X-ray fluorescence spectrometry: an example of simultaneous determination of Fe, Co, Zn, Ga, Se and Pb in water samples, Journal of Analytical Atomic Spectrometry, 26 (2011) 1979-1985 mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w niniejszej pracy oraz redakcji manuskryptu.

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Oświadczenie

Oświadczam, że w pracy R. Sitko, K. Kocot, B. Zawisza, B. Feist, K. Pytlakowska, Liquid-phase microextraction as an attractive tool for multielement trace analysis in combination with X-ray fluorescence spectrometry: an example of simultaneous determination of Fe, Co, Zn, Ga, Se and Pb in water samples, Journal of Analytical Atomic Spectrometry, 26 (2011) 1979-1985 mój udział polegał na przeanalizowaniu wpływu średnicy analizowanej próbki na natężenie promieniowania i dyskusji wyników.

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Oświadczenie

Oświadczam, że w pracy R. Sitko, K. Kocot, B. Zawisza, B. Feist, K. Pytlakowska, Liquid-phase microextraction as an attractive tool for multielement trace analysis in combination with X-ray fluorescence spectrometry: an example of simultaneous determination of Fe, Co, Zn, Ga, Se and Pb in water samples, Journal of Analytical Atomic Spectrometry, 26 (2011) 1979-1985 mój udział polegał na wykonaniu pomiarów techniką ETAAS.

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Oświadczenie

Oświadczam, że w pracy R. Sitko, K. Kocot, B. Zawisza, B. Feist, K. Pytlakowska, Liquid-phase microextraction as an attractive tool for multielement trace analysis in combination with X-ray fluorescence spectrometry: an example of simultaneous determination of Fe, Co, Zn, Ga, Se and Pb in water samples, Journal of Analytical Atomic Spectrometry, 26 (2011) 1979-1985 mój udział polegał na dyskusji wyników dotyczących precyzji opracowanej metody.

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Oświadczenie

Oświadczam, że w pracy K. Kocot, B. Zawisza, R. Sitko, Dispersive liquid-liquid microextraction using diethyldithiocarbamate as a chelating agent and the dried-spot technique for the determination of Fe, Co, Ni, Cu, Zn, Se and Pb by energy-dispersive X-ray fluorescence spectrometry, SpectrochimicaActa Part B, 73 (2012) 79-83 mój udział polegał na analizie statystycznej wyników związanych z dokładnością metody mikroekstrakcji powiązanej z techniką dried-spot i analizą EDXRF.

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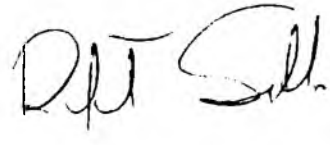
Oświadczenie

Oświadczam, że w pracy K. Kocot, B. Zawisza, R. Sitko, Dispersive liquid-liquid microextraction using diethyldithiocarbamate as a chelating agent and the dried-spot technique for the determination of Fe, Co, Ni, Cu, Zn, Se and Pb by energy-dispersive X-ray fluorescence spectrometry, Spectrochimica Acta Part B, 73 (2012) 79-83 mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w niniejszej pracy.



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Oświadczenie

Oświadczam, że w pracy K. Kocot, B. Zawisza, E. Margui, I. Queralt, M. Hidalgo, R. Sitko, Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples, Journal of Analytical Atomic Spectrometry, 28 (2013) 736-742 mój udział polegał na zaplanowaniu doświadczeń dotyczących procedury zatężającej i interpretacji wyników uzyskanych dla próbek wód.

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DECLARATION

I declare that my contribution to the paper K. Kocot, B. Zawisza, E. Margui, I. Queralt, M. Hidalgo, R. Sitko, Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples, Journal of Analytical Atomic Spectrometry, 28 (2013) 736-742 consisted on the performance of part of the experimental work (the results presented in Fig. 4) and revision of the manuscript.

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DECLARATION

I declare that my contribution to the paper K. Kocot, B. Zawisza, E. Margui, I. Queralt, M. Hidalgo, R. Sitko, entitled "Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples", published in the Journal of Analytical Atomic Spectrometry, 2013, 28:736-742, consisted in the assessment and selection of the best conditions for the improvement of analytical response of TRXRF instrumentation to obtain the best performance of the proposed methodology. The analytical tasks were made at the joint laboratory facility LARX-ICTJA/University of Girona.

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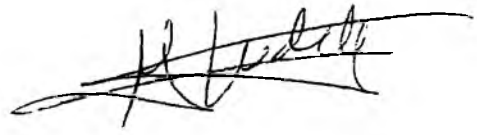
DECLARATION

I declare that my contribution to the paper K. Kocot, B. Zawisza, E. Margui, I. Queralt, M. Hidalgo, R. Sitko, Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples, Journal of Analytical Atomic Spectrometry, 28 (2013) 736-742 consisted in collaborating with the other co-authors in data interpretation and results discussion as well as in the critical revision of the manuscript.

Signature



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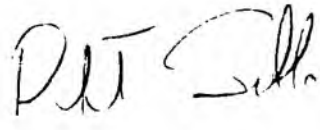
Oświadczenie

Oświadczam, że w pracy K. Kocot, B. Zawisza, E. Margui, I. Queralt, M. Hidalgo, R. Sitko, Dispersive micro solid-phase extraction using multiwalled carbon nanotubes combined with portable total-reflection X-ray fluorescence spectrometry for the determination of trace amounts of Pb and Cd in water samples, Journal of Analytical Atomic Spectrometry, 28 (2013) 736-742 mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w niniejszej pracy.



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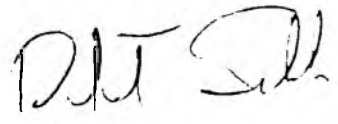
Oświadczenie

Oświadczam, że w pracy K. Kocot, R. Sitko, Trace and ultratrace determination of heavy metal ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid sorbent in dispersive micro solid-phase extraction, Spectrochimica Acta Part B 94-95 (2014) 7-13 mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w niniejszej pracy.



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Genoa, December 23, 2014

DECLARATION

I declare that in the paper K. Kocot, R. Leardi, B. Walczak, R. Sitko, Determination and speciation of trace and ultratrace selenium ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid adsorbent in dispersive micro-solid phase extraction, Talanta, 134 (2015) 360-365 my contribution was related to the set up of the experimental design, the elaboration of the resulting data and the revision of the manuscript.

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Oświadczenie

Oświadczam, że w pracy K. Kocot, R. Leardi, B. Walczak, R. Sitko, Determination and speciation of trace and ultratrace selenium ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid adsorbent in dispersive micro-solid phase extraction, Talanta, 134 (2015) 360-365 mój udział polegał na kierowaniu projektem naukowym obejmującym badania opisane w niniejszej pracy.